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# YEAR BOOK 1955-1956

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# CONTENTS

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	PAGE
OFFICERS AND STAFF	v
REPORT OF THE PRESIDENT	1
REPORTS OF DEPARTMENTS AND SPECIAL STUDIES	
Mount Wilson and Palomar Observatories	31
Joint Committee on Image Tubes for Telescopes	65
Department of Terrestrial Magnetism	69
Geophysical Laboratory	153
Department of Plant Biology	231
Department of Embryology	269
Department of Genetics	293
Department of Archaeology	335
BIBLIOGRAPHY	343
REPORT OF THE EXECUTIVE COMMITTEE	I
Report of Auditors	III
ABSTRACT OF MINUTES OF THE FIFTY-EIGHTH MEETING OF THE BOARD OF TRUSTEES	XIX
INDEX	XXI





# PRESIDENT AND TRUSTEES

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Mount Wilson Observatory organized in 1904; George E. Hale, Director 1904–1923, Honorary Director 1923–1936; Walter S. Adams, Director 1924–1945. Unified operation with the Palomar Observatory of the California Institute of Technology began in 1948.

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2801 UPTON STREET, N.W., WASHINGTON 8, D. C.

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5241 BROAD BRANCH ROAD, N.W., WASHINGTON 15, D. C.

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F. T. MCCLURE  
G. F. PIEPER  
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\* Retired in 1956.

‡ On leave of absence.

§ Resigned in 1956.

|| Transferred to Geophysical Laboratory in 1956.

¶ Term of appointment completed in 1956.

## DEPARTMENT OF PLANT BIOLOGY

STANFORD, CALIFORNIA

Desert Laboratory, opened in 1903, became headquarters of Department of Botanical Research in 1905; name changed to Laboratory for Plant Physiology in 1923; Daniel T. MacDougal, Director 1906-1927. Reorganized in 1928 as Division of Plant Biology, including Ecology; Herman A. Spoehr, Chairman 1927-1930 and 1931-1947, Chairman Emeritus 1947-1950. Name changed to Department of Plant Biology in 1951.

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WOLFE AND MADISON STREETS, BALTIMORE 5, MARYLAND

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COLD SPRING HARBOR, LONG ISLAND, NEW YORK

Station for Experimental Evolution opened in 1904; name changed to Department of Experimental Evolution in 1906; combined with Eugenics Record Office in 1921 to form Department of Genetics. Charles B. Davenport, Director 1904-1934; Albert F. Blakeslee, Director 1935-1941.

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10 FRISBIE PLACE, CAMBRIDGE 38, MASSACHUSETTS

Department of Historical Research organized in 1903; Andrew C. McLaughlin, Director 1903-1905; J. Franklin Jameson, Director 1905-1928. In 1930 this Department was incorporated as a section of United States history in a new Division of Historical Research; Alfred V. Kidder, Chairman 1930-1950. Name changed to Department of Archaeology in 1951.

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JOHN VON NEUMANN, Institute for Advanced Study  
HANS RAMBERG, University of Chicago  
C. E. TILLEY, Cambridge University

\* Resigned in 1956.

† Term of appointment completed in 1956.

§ On leave of absence.

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CARNEGIE INSTITUTION OF WASHINGTON

REPORT *of*

THE PRESIDENT



## REPORT OF THE PRESIDENT

THIS is the fifty-fourth year in the life of the Carnegie Institution of Washington. It is two years beyond a half century since, under its new Articles of Incorporation, the Institution's affairs were placed under the control of a Board of Trustees and it assumed essentially its present form and function. In the intervening fifty years there have been minor adaptations of form and there has been an extraordinary enlargement of scope and of significance, but there has been remarkably little change in the structure or the basic orientation of the Institution.

By any American standard, fifty years is a long span. By the standards of American scientific research, it is an epoch. When the Carnegie Institution was founded, it is not too much to say that in America even the idea, let alone the practice, of scientific research in its investigational, nonengineering sense had scarcely been born; and the notion of scientific investigation except as an integral but subsidiary part of university work was so strange as to be unpalatable. An archive which the Institution may properly cherish is an editorial in *Science* for 1903, expressing the gravest doubts about the function or the future of an organization devoted to such an idea. The industrial laboratory was not to appear in America in its modern form for some five more years, and it would be at least another twenty before that pattern of research would become common. To be sure, a few internationally eminent men of science were at work in American universities: Josiah Willard Gibbs at Yale, Alexander Agassiz at Harvard, himself an early Trustee of the Carnegie Institution, and the remarkable group already assembled at the Johns Hopkins come readily to mind. But in the world scene American science had a decidedly minor place, and in magnitude it was relatively insignificant.

In such a setting an organization endowed with the generosity of Mr. Carnegie's gift and devoted to the furtherance of scientific investigation could hardly fail to have a major impact, for good or ill. It lay within the Institution's power to change an already existing trend in American science, or to initiate a new one, simply by a direction of substantial funds. Strokes of genius and serious errors of conception and planning alike could be multiplied an hundredfold.

It is remarkable that in the face of this grave responsibility, with little American tradition for guidance except that provided very indirectly from the universities, a plan of operation for the Institution was devised and exe-

cuted in 1904 which seems in retrospect to have been uniquely effective among the possibilities that were open. The plan embodied three decisions that were to set the whole character of the Institution: the decision to direct its activities to investigation as distinct from teaching, except for such incidental teaching activity as might further the general research effort; the decision to make of the Institution primarily a "working" rather than a granting body; the decision to organize the work of the Institution into departments, geographically dispersed through the country and so placed as to have ready access to a number of the leading universities. The early history of Institution activity has left no doubt of the extraordinary wisdom of these choices. It is interesting and important to consider how well they have weathered an almost incredible half century of scientific development in this country, and how relevant they are today.

It is hard to imagine more radical changes than those in the American scientific scene since those early days of the Institution. There have been two major wars, in each of which American survival was at stake. The first was America's real introduction to the place of technology in warfare. The second witnessed that revolution in the concept of the nature of national security and the role of technology in the defense of the free world which—but ten years later—is so universally accepted today. Never, in terms of its basic philosophy, its tradition, its mode of work, or its organization, has the Institution been designed for or adjusted to applied research; yet it was able to play an important part in the technological change. In the First World War it made major contributions to American military research and planning by virtue of its fund of basic knowledge, its flexible form of organization, and its will. In the Second World War, the Institution, through the vision and the action of its President and the Office of Scientific Research and Development, served as the home of the greatest innovations of our time in scientific organization and execution for war and defense. On the technical side, some of the most important ideas in modern weaponry derive from those years. On the organizational side, a considerable number of the great contemporary government organizations for science take their genesis from this same period and in this same activity. It would be hard to find a milieu farther removed from the original world or conception of the Institution in 1904 and 1905. And yet the large decisions taken then stood the test of national emergency, and stood it well. What of the greater test of the non-crisis years, when the Institution was freed for its own proper task?

The change involved in transition from a world of free scientific investigation to an emergency one of developmental technology, rigidly applied under the time scale and the ruthlessly uncompromising conditions dictated by the challenge of survival itself, was severe and radical enough. But I doubt whether it exceeded the contrast between the scene of normal uncommitted

American scientific research in 1906 and that in 1956. Scientific leadership is an intangible and protean thing, difficult to evaluate at any moment and, because of the nature of research itself, ever changing from year to year. But it is probably fair to say that in 1956 American science, considering its quantity and quality together, may lay claim to world leadership. In the universities, in multifarious industrial research laboratories, in government, scientific research is being prosecuted in quantity and quality such as the world, let alone our own nation, has never known before. It is not only that with the explosive expansion of university facilities which is in full course today we are making better use of the native abilities of our younger population and may expect to do still better in the future, and thus have greatly broadened the base from which American scientific talent may come. It is not only that we have benefited incalculably in recent years from those brilliant minds of Europe and of Asia that have chosen to make America their home. The most conspicuous element of all, perhaps, is a quality inherent in the structure of science itself which has come to prominence in these years. It is the exponential character of the rate of accretion of information when not only the techniques of seeking new knowledge are developed, but also systematic means for formulating the very questions by which new knowledge shall be sought and found are devised. And we are full-tide, now, in this explosive phase.

Against this vast and dynamic background the Institution continues to pursue its aims of research toward the same goals, and essentially in the same manner, as it has throughout its working life. It can bring far greater stores of experience, and in absolute terms even slightly augmented material resources, to the task. Over the immense transition from peace to war—from an isolated America, almost a frontier land in her concept of science, secure in the protection of a British navy, two vast and relatively invulnerable seas, and a waste of empty sky, to an embattled America deep in a predominantly technological struggle for her very life—the organization and the traditions and the purposes of the Institution were such as to allow it to play a significant role. Is this also true of the yet greater transition that a half century of science, and a major transformation in the scale and structure of American society at peace, have wrought? There are many reasons for believing that those goals for the Institution, and even the broad manner of achieving them, which were selected and established fifty years ago are as relevant to leadership in the present scene as they have ever been. Let me examine some of them.

The Institution is dedicated to the ideal of uncommitted scientific investigation, to the scholarly search for scientific truth wherever it may lie, without primary consideration of its use in any practical sphere. Such use, of course, is neither precluded nor to be ignored. Many practical “firsts” in



American technology have come from this sort of uncommitted investigation in the Institution: hybrid corn, the basis of modern cement manufacturing methods, important innovations in refractories for steel plants, the initiation of Pyrex glass, the first intimations of the principles of radar, to name but a few. Yet, important as they are in a practical sense, they are incidental to the main objectives of the Institution programs, and primarily illustrative of the widespread paradox that the most important practical consequences are commonly the least sought after.

The Institution's dedication to the role of the uncommitted investigator has further corollaries which are of real importance in the current American scientific scene. It implies dedication to investigatory work of the highest quality and takes as its objective, among other things, the maintenance of research standards. It implies great flexibility, a willingness to ignore the boundaries of established fields and to seek knowledge in those rich borderline areas that so often lie between them, a readiness to ignore conventional limitations and to follow the truth where it may lead. Above all, it implies a resolution to foster and to pay tribute to that kind of inspiration and sense of fitness in creative thinking, to the ideal of balance and proportion, which in other fields has sometimes been called a "sense of style." These are precious goals.

A series of investigations has recently been undertaken in England by Derek J. Price, seeking to gain some measure of the rate of increase of the volume of scientific effort. The criteria were chosen somewhat arbitrarily and partly for their manageability, but they embraced a series of generally related quantities, such as the total of registered scientific manpower, the annual expenditure on scientific matters, the number of scientific patents, the volume of scientific journals. These are admittedly rough and often partially erroneous criteria, and they give little indication of quality. The interesting thing, however, is that nearly all of them show the same general growth trends, and that these trends are essentially exponential. Moreover, the constant of the exponential curve is found to effect a doubling of the volume of scientific effort in approximately every ten to fifteen years. This is not basically a new phenomenon. There is evidence that the total amount of the scientific effort has been increasing exponentially since the time of Newton. But, as Price points out, the present rate of doubling for the total magnitude of scientific effort—two or perhaps even three times in a generation of men—is appreciably greater than that for many nonscientific and nontechnological activities. There is no reason to assume that the rate of growth of scientific effort will change from exponential to linear in the immediate future.

Among the many implications of this situation, two seem of particular importance to the present and future role of the Carnegie Institution of Washington. The first is one specifically pointed out by Price, though gen-

erally long recognized in many fields. When an attempt was made to evaluate the rate of increase in scientific attainment of the highest quality (and admittedly the yardsticks are exceedingly intangible here), it developed that this rate was considerably lower than that for the volume of research as a whole. As Price estimates in his English survey, its volume takes about three times as long to double. In this country reliable comparison is almost impossible because of the difficulty of definition. It seems likely, however, that the discrepancy is if anything greater because of the American tendency, especially since the Second World War, to label a great deal of technological effort as research which is in fact engineering development. Thus the field of uncommitted research of the highest quality, with which the Institution is primarily concerned, is both very much smaller and of slower rate of growth than the scientific effort as a whole. This means, of course, that the Institution remains, in a purely material sense, a larger factor in this field than in the wider one. But there is a much more important and more relevant aspect. In science as in other creative endeavors the small percentage of innovational effort of the highest quality carries significance both in its own field and in society as a whole far beyond its absolute magnitude. It is therefore peculiarly important. It is also especially vulnerable, for, however much its merit for the future may be recognized, it must always wage a continuing struggle to maintain its contemporary integrity, partly because of its deficiencies of magnitude, partly because the motivations of such work, spiritual and material, are not easily communicated at the time when it is in progress. And so the dedication of the Institution to standards of the highest quality in research, and research people, constitutes an important leadership function for the present and the future.

A second implication has to do with scientific specialization and its consequences. The antithesis between wisdom and knowledge is a truism. Yet it comes home with peculiar force in respect to scientific matters. The pressures that make for specialization in science are many and compelling. Specialization, in a very real sense, is the life blood of scientific investigation. Only the specialist, the investigator who has spent years with his limited subject in its most intimate detail, whose mind and whose eye and whose whole being are attuned to its peculiar subtleties, is in a position to grasp the opportunities that lie deeply hidden, to achieve the truly significant insights. Except for the occasional genius of the caliber of a da Vinci, it is only to such men that real penetration can come. It is still as it was said by Pasteur: "In experimentation, chance favors only the prepared mind." This is perhaps the most compelling, and indeed the most essential, of the reasons for scientific specialization. Other reasons are scarcely less important. It is only to those who have won mastery of their subject that the full satisfaction and joy of creativity belong. It follows that these rewards, the most power-

ful incentives to research, can come only to him who, in some measure at least, is a specialist. And to the specialist in the company of his fellows belong those other rarest of working rewards: encouragement, reinforcement, solidarity, the powerful spirit of a truly understanding working fraternity.

But the pernicious effects of unchecked specialism are notoriously legion. As specialties diverge and grow—and their rate of divergence is in keeping with the rate of growth of science as a whole—they become vastly remote from one another, as disparate as the points of the compass. In the early stages this divergence may be one principally of language. But in actively growing fields the alienation quickly extends to the very concepts for which the words stand. In the end, divergent specialties may differ as much, in language, in mode of approach, in basic orientation, in systems of values not only about their own subject but about the world in general, as two different cultures. Once these differences are firmly established, strong barriers arise to the crossing of boundaries. The specialist now is professionally identified with his specialty. It is but human to adhere to it tenaciously in virtue of its own name and rubric, and to be hostile to other areas of knowledge that are now not so easy to understand or to appreciate. The whole matter of professional identification with other members of his specialty as distinct from workers in adjacent fields, in postgraduate and in later professional life, adds a final practical compulsion to the isolation of the specialist. To a dangerous degree, he has sacrificed his flexibility. And so is maintained and ever emphasized that bane of our times which follows so naturally from the accretion of knowledge, which is so antithetical to true wisdom, of which we are so conscious but about which we have yet been able to do so little—excessive fragmentation.

There is much evidence of a widespread growing realization of this peril, and there is a great need for leadership in combating it. Specialization can never be avoided; it is a keynote of scientific expansion and of our technological civilization. But the ideal can be emphasized of the superior individual whose capacity, whose training, whose judgment, and whose orientation will be those of the generalist, without loss of the discipline or the competence of the specialist. It is not an unattainable ideal. Men of high quality and aspiration have reached it before.

The Carnegie Institution, by virtue of its tradition and its structure, is particularly well placed to give leadership here. It has always been a fundamental part of the Institution's policy that the subject areas in which it worked should be chosen with great flexibility, that the rubrics of the departments should not be taken rigidly to restrict their subject matter, that investigations of areas considered by competent men to be of great inherent interest should be furthered whether they fell within conventionally recognized boundaries of scientific disciplines or squarely between them, that every encouragement



should be given to the taking of long intellectual risks by those equipped to do so. Of recent years some of the most exciting and profitable investigations in the Institution have fallen between the fields of physics and astronomy, or of biology, chemistry, and physics. It has always been Institution policy that the work of its staff should be evaluated in terms only of the mature and collective judgment of their peers, and never by those conventional standards which too often tend to solidify the boundaries of specialties and break down understanding between them. There is an important task to be performed both in demonstrating that specialization is the necessary servant, but must not be the master, of a discipline, and in training future leaders in scientific research to whom this truth is familiar and accepted.

These are the answers to the question whether the goals and the method of approaching them which were established for the Carnegie Institution more than fifty years ago are still relevant today. The functions of the Institution in the coming years must be what they have always been: to stimulate scientific research of the highest caliber, to conceive and open new areas of research which can later be taken up by others, to provide advice and guidance in scientific matters at the most effective levels of national policy, but, above all, to enrich the national resources of research personnel and the conception of research standards and their goals. Once it was the relative amount of Institution activity that counted most heavily. Now it is a matter of philosophy, and of quality. And here, once again, the first key decisions stand valid.

In such a context the Institution must remain, as it has been throughout its life, primarily an investigative and primarily a working body. For no group that aspires to set standards in the scientific community is entitled to respect or can carry conviction unless its own house demonstrates actively and unequivocally those standards to which it is dedicated.

This is the root from which many other means of leadership and of communication, chosen flexibly as circumstances dictate, may stem. The dispersion of the departments of the Institution and their proximity to leading universities, both geographically and in fields of work, offer great opportunities in many important sectors of scientific activity other than substantive research. But the whole effort will have validity, and conviction, only to the degree that it remains firmly based in the programs of uncommitted research of high quality and fresh interest going forward within the Institution. They are the essence of a very precious way of life.

## THE YEAR'S WORK IN REVIEW

### *The Scientific Program*

It is always difficult to select individual researches of the year for special mention. It may be misleading to do so without emphasizing that the programs so selected are taken only as examples of the kind of work that has

been going forward in the various departments during the year. Their mention in this summary review implies only their representative interest; it cannot imply that they are either more or less striking or important than many other programs that might have been described. The reader who is especially interested in the work of any department should turn to the fuller review of its work later in the report.

This year marks a point of particularly vivid interest in the programs of the Mount Wilson and Palomar Observatories which are concerned with the nature and extent of the universe. In the second decade of the century, W. M. Slipher, at the Lowell Observatories, measuring the spectral redshifts of a number of stellar objects, showed that they were receding from us at great speeds. Edwin P. Hubble, at Mount Wilson, establishing that these objects were actually systems of stars, measured the distances of many of these nebulae and demonstrated not only that some of them lay at far greater distances from us than had been imagined, but that their velocities of recession, as measured by their redshifts, increased strictly in proportion to their distances. This was the basis for Hubble's now classic hypothesis that the universe as a whole is expanding, and his prediction that more remote galaxies, as they were examined, would show yet larger redshifts, and in proportion to their distances. Ever since that time, study of this phenomenon has continued, especially by Humason at Mount Wilson and Palomar. In 1936 the limit of range of the 100-inch telescope was reached, and Hubble's prediction was fulfilled: all the way out the redshift increased in proportion to the distance, suggesting a uniformly expanding universe. When the 200-inch telescope became available at Palomar, the program was resumed with new spectrographic equipment. Redshift measurements of clusters of galaxies with velocities of recession up to 60,000 kilometers per second have now been accomplished. Far out, the linear proportion held. But clusters about a billion light-years removed are found to be moving faster than in direct proportion to their apparent distance. If confirmed, such data can be interpreted to mean that a billion years ago the universe was expanding *more rapidly* than it is at present. To test this further, the current redshift program is confined to observation of faint galaxies in the most distant clusters that can be found and observed—a difficult technical achievement. Two such clusters, with predicted redshifts greater than 100,000 kilometers per second, have already been observed by Humason, and measurements of the spectra indicate that the shifts for both are probably large and of the right order. If the measurements and interpretation are verified, they suggest that our universe is an evolving rather than a steady-state one. They may allow conclusions of a fundamental and far-reaching nature about the geometry of space, and about the mean density of matter in the universe. The present observations,

for example, are in conflict with the concept of a steady-state universe as proposed by Hoyle and by H. Bondi and T. Gold. They require an expanding model of finite dimensions and with a positive radius of curvature. The expansion will eventually stop, and contraction will set in. The universe is of the so-called oscillating type. It may well be that the studies begun by Hubble and Humason three decades ago will reach a most important, and indeed most dramatic, climax in the next year or two.

A second program of great interest at the Observatories is concerned with problems of stellar evolution. How are stars formed? What is their lifetime? How do various observable properties, such as absolute brightness, surface temperature, and chemical composition, change during this lifetime? According to present concepts, stars are formed originally by the condensation of clouds of material, principally hydrogen. As the cloud contracts into a star, its core heats to temperatures at which transformation of the hydrogen to helium takes place. This nuclear fusion reaction produces very large quantities of energy, which in turn are radiated away at the surface of the star. A point is soon reached at which the generation of energy is just balanced by radiation loss. In this initial steady state both the brightness of the star and its surface temperature vary sharply with the mass. A star with a mass twelve times that of the sun gives off 1000 times its light and has a surface temperature of  $23,000^{\circ}\text{C}$ —four times that of the sun. At the other extreme, a star with a mass one-seventh that of the sun radiates at one-tenthousandth of the rate and has a surface temperature of only  $3000^{\circ}\text{C}$ . Stars that are in this initial state of equilibrium between energy generation and energy loss through radiation, and which maintain this relation between mass, luminosity, and surface temperature, are said, in evolutionary terms, to be on the *main sequence*.

As time goes on, the hydrogen fuel of these stars is depleted. Eventually their composition becomes so changed that the equilibrium of energy generation and dissipation is upset. Theory predicts that, when this occurs, the star will slowly expand, its surface will cool, and it will appear redder. At the same time its rate of energy radiation will actually increase. Then, as the hydrogen fuel is still further exhausted, the process is reversed, the star contracts and grows hotter, but also fainter. At one or more stages in this evolution the star becomes unstable and pulsates rapidly with a corresponding oscillation in brightness. Eventually, when the fuel is completely exhausted, the star stops radiating and can no longer be observed.

This picture of stellar evolution provides an explanation for the observations on different types of stellar populations which have been carried forward by Baade for a number of years and have been reported in previous Year Books. Thus, for example, huge clouds of gas and dust are observed in the spiral arms of our own Galaxy and of the Andromeda galaxy, where



Population I stars predominate. These clouds are still condensing to form stars, and in these arms young, comparatively newly formed stars abound. The most characteristic features of such "young" populations are very massive, exceedingly bright, and very hot blue stars.

By contrast, in the central regions of the galaxies, where Population II stars predominate, the stars must be comparatively "ancient," having been formed billions of years ago from dust or gas clouds which have now completely vanished. All the very bright blue stars have used up their fuel and disappeared; they are not represented in these "old" populations.

It is evident that there will be a difference in the rate of evolution between the very bright stars, which are using up their hydrogen fuel rapidly, and the faint stars, which are using theirs at a slower rate. The brightest stars of the old Population II groups, therefore, are the stars of intermediate brightness that have just reached the stage where they expand and cool but at the same time become brighter. The most conspicuous stars in this population are therefore cool and red.

This is the broad picture suggested by Baade's discoveries of Population I and Population II. But many parts of the theories must be refined and filled in, and extremely active work is in progress, theoretical and observational, both at the Institution and at the California Institute of Technology, to this end. One example of many such researches currently in progress at the Observatories is offered by investigations on the relations between absolute brightness and surface temperature, as indicated by the color index, for stars in a number of clusters. A comparison of the shape of, and distribution along, the color-magnitude diagram thus obtained with the color-magnitude diagram of the main-sequence stars yields information on the manner in which the properties of stars of various masses change with age, and the rate of such change. Extensive spectroscopic investigations are in progress on main-sequence stars and also on stars in the globular clusters to determine their chemical compositions for comparison with the predictions made from laboratory studies of reaction rates. The combined result of all these laboratory, theoretical, and observational studies is leading to a greatly expanded understanding of stars, the source of the energy they radiate, and their evolutionary history.

The year has also been a profitable one in the new field of the study of stars as radio sources. One of the outstanding events of last year was the explanation of the light emission in the Crab Nebula (the remains of a supernova, the explosion of which is recorded in Chinese annals in A.D. 1054) as due to electrons circling in a magnetic field—a situation similar to that in the synchrotron and so known as the synchrotron mechanism. Since, according to this theory, strong polarization (up to 100 per cent) was to be expected in the continuum of the Nebula, a test at the 200-inch telescope

seemed especially desirable. Measurements of such effects made by Baade reveal marked polarization, in accordance with theory, and this finding has been confirmed by Baum and by Zwicky, using different methods. Similar confirmations of theory are suggested in another celestial object in which the synchrotron mechanism is believed to be operative: the jet in the M 87 galaxy. Both the Crab Nebula and M 87 are strong sources of radio noise and have continuous, as opposed to emission, optical spectra. These conditions are theoretically explicable on the basis of the synchrotron mechanism.

The present year has seen the completion of the National Geographic Society-Palomar Observatory Sky Survey, generously financed by the National Geographic Society. The whole sky accessible from Palomar Mountain has been photographed in both red and blue light, a labor requiring for its completion almost the whole observing time of the 48-inch schmidt camera for seven years and using 879 plates in each color. The blue plates reached a limiting magnitude of 21.1, two or three magnitudes beyond that of any previous survey of the whole sky, allowing an increase in this survey by a factor of about 3 in depth penetrated and of about 25 in total volume of space recorded. Many new objects of great potential interest have been found, the full study of which will require many decades.

Of more intimate interest to us than the ages and rates of evolution of the stars in distant galaxies is the age of our own earth. One clue to the determination of the age of the earth comes from the measurement of radioactive decay in ancient rocks. Early in the century, the radioactive decay of uranium and thorium was recognized as a useful yardstick in making such measurements. It presented certain difficulties, and in more recent years other radioactive decay chains have been investigated, offering widened possibilities for correlation and for checking.

For several years past, the staffs of the Department of Terrestrial Magnetism and the Geophysical Laboratory have joined in a critical study of such methods of measuring the ages of minerals. Special attention has been given to the new methods made available by the natural radioactive decay of rubidium (to strontium) and of potassium (to argon).

It has been found that for pure mica crystals separated from almost any igneous rock, the two ages obtained from the decay of potassium and of rubidium agree within 10 per cent. Further, when it is possible to determine unambiguously the age of the rock from the radioactive decay of uranium to lead, the potassium-argon and the rubidium-strontium figures for the mica agree with the uranium-lead ages. For many igneous rocks the ages from the decay of uranium to lead do not agree. In all such cases that have been studied, it is found that the potassium-argon and rubidium-strontium ages of mica from these rocks still agree. It looks very much, therefore, as though

accurate ages may now be obtained for many geologic formations which previously could not have been measured. Indeed, the new methods for dating igneous intrusives and inclusions in ancient sediments may make it possible to place even these formations in an absolute time sequence. World-wide correlations of pre-Cambrian sedimentary formations would then become a real possibility for the first time. The methods have already established that sedimentary and volcanic formations older than 2500 million years occur on the North American, Australian, and African continents.

Changes in the nature of the earth's crust with increasing depth have long been of interest both to the Department of Terrestrial Magnetism and to the Geophysical Laboratory. For some years past the Department of Terrestrial Magnetism has conducted a series of seismic investigations in the field, recently ranging as far as the Alaskan peninsula, to investigate interesting and significant crustal discontinuities. Parallel laboratory experiments have been continued at the Department by Tatel and his associates. Recently a very interesting new approach to the whole problem of the nature of underlying causes of the Mohorovičić discontinuity has been suggested by work at the Geophysical Laboratory. A study of phase changes occurring in certain rock minerals under conditions of temperature and pressure simulating those obtaining under natural conditions in the vicinity of the Mohorovičić discontinuity suggests that the discontinuity may be primarily the result of sharp phase boundaries, sensitive to small shifts of temperature and pressure, in that region. This is a very different concept from the older one that the discontinuity is primarily the result of changes of chemical composition with increasing depth. So revolutionary may its implications be, in fact, in relation to such matters as the mechanisms of mountain building, that extensive further laboratory investigation of these phase-shift phenomena seems urgent. Accordingly, phase changes have been studied this year at the Geophysical Laboratory at pressures as high as 60,000 atmospheres and temperatures up to about 700° C, using equipment built by Boyd and England. With this apparatus dense minerals have been artificially produced such as might exist naturally deep in the earth. Exploratory experiments of this kind have been conducted on feldspars, feldspathoids, and denser minerals. Transitions have been found to occur in nepheline, leucite, and anorthite. Anorthite is particularly interesting, since it is common as a component in plagioclase in deep-crystallizing plutonic rocks, and its breakdown deep in the crust might well contribute to the Mohorovičić discontinuity.

Continuing his investigation of the composition of minerals in the earth's crust, Eugster at the Geophysical Laboratory has synthesized pure iron-bearing mica annite and has opened new areas of opportunity in mineral synthesis. He has determined the field of stability of this mica as a function of tem-



perature, oxygen pressure, and water pressure, and has shown that even a small increment of oxygen pressure may drastically lower the breakdown temperature of the mineral.

The first determinations of stability of a sulfide mineral under pressure have been made by Kullerud and Yoder. Using a new technique of general applicability, they have studied the upper stability curve of pyrite ( $\text{FeS}_2$ ). It proves to be stable below  $815^\circ\text{C}$  at a pressure of 5000 bars. These experiments help to explain why primary pyrite occurs in deep-seated granites and amphibolites but not in basalts or in rhyolites.

Varied fundamental investigations of the nature of life and life processes continue to attract the attention of five departments of the Institution: the Department of Terrestrial Magnetism, the Geophysical Laboratory, and the Departments of Genetics, Plant Biology, and Embryology. At the Geophysical Laboratory, Abelson, continuing his pioneer work on the existence and nature of very ancient and primitive life, has shown that amino acids such as glycine, sarcosine, alanine, and  $\beta$ -alanine can be formed from a variety of "primitive atmospheres." Simulating conditions that might have been present in the early history of the earth, he has subjected gaseous mixtures to electrical discharges. Among the combinations yielding amino acids were  $\text{CO}_2\text{--N}_2\text{--H}_2\text{--H}_2\text{O}$ ,  $\text{CO--N}_2\text{--H}_2\text{--H}_2\text{O}$ ,  $\text{CO}_2\text{--NH}_3\text{--H}_2\text{--H}_2\text{O}$ , and  $\text{CH}_4\text{--N}_2\text{--H}_2\text{O}$ .

Studies of certain fundamental aspects of metabolism in microorganisms are continuing at the Department of Terrestrial Magnetism. From the beginning, these studies focused about the problems of protein and nucleic acid synthesis at the molecular level in intact cells growing exponentially in synthetic media. Two very different types of microorganism have been used in this work, contrasting greatly in complexity—the bacillus *Escherichia coli* and the yeastlike *Torulopsis utilis*. For several years the studies were concentrated on the synthesis of small molecules by these organisms: amino acids, purines, and pyrimidines, which serve as building blocks for the proteins and nucleic acids. Last year and this, investigation has progressed to a study of the ways in which these blocks are incorporated.

In general, the first step in such synthesis is for the cell to concentrate the small molecules into a relatively loosely held "pool," where they are retained and subsequently linked into the macromolecules. The past year's work has greatly increased our knowledge of the characteristics of these pools. In addition, some of the immediate precursors of nucleic acid have been determined, and an upper limit for the time required for protein synthesis has been established. It is three seconds.

Studies of these pools of synthesis for proteins and nucleic acids have been of direct interest to the investigations in bacteriophage metabolism at the Department of Genetics. Here, however, interest has been centered primarily

on the intact nucleic acid in the phage itself, on its mode of transfer, and on its role in heredity.

Previous work by Hershey and his collaborators, utilizing radioactive tracers, had shown that the main body of a bacterial virus particle is made up of deoxyribonucleic acid and that the coat surrounding the body contains protein. These workers had also demonstrated that the DNA transmits viral hereditary characteristics. During the past year they have obtained evidence from two new experimental sources that large, functionally intact pieces of viral nucleic acid may pass from parent to offspring phage. If bacteria are infected with a mixture of ultraviolet-irradiated and nonirradiated phage particles, a small number of noninfective offspring particles is produced. These particles contain a high concentration of nucleic acid derived from the irradiated parent. Thus radiation-induced lesions must be transmitted from parental to offspring particles in the form of large pieces of irradiated parental nucleic acid. Again, if bacteria are infected with two kinds of phage particles, differing both in their genetic markers and in their labeled phosphorus, about two-thirds of the parental phosphorus remains associated with the parental genotype during transfer from parent to offspring phage. Both results suggest that part of the viral nucleic acid is contained in a functional chromosome that is not subject to progressive fragmentation during replication and genetic recombination.

A bacterial cell is much more highly organized than a bacterial virus particle. Powerful biological methods have been developed, however, for investigating the genetic properties of certain simple unicellular organisms. One of the most sensitive of these makes use of the phenomenon of "transduction." This is the transport of fragments of genetically active material, presumably small segments of chromosome, by infective virus particles, from the bacterium in which the phage have been raised into the bacteria which they subsequently infect. Demerec and his collaborators have used this method to carry out penetrating genetic analyses of short regions of the chromosome complex of the bacterium *Salmonella typhimurium*. Their studies have demonstrated that the "gene" as previously defined is not the ultimate hereditary unit, but can be broken down with these finer analytical tools into yet smaller entities (sites), linearly arranged in a definite order. During the past year it has been shown that mutations occurring at different sites of a single locus, although they affect one function primarily, may produce mutants that differ considerably from one another with respect to various properties.

Several years ago McClintock demonstrated the existence of certain heritable units in the chromosome complement of maize that served to control and modify the effects of other genes, without themselves directly affecting the phenotype. Knowledge of such elements has been considerably expanded during the past year. A number of these units, now called "controlling ele-



ments," have been identified and their distinctive modes of operation examined. It is now evident that they may act not only as mutators, but also as inhibitors, suppressors, enhancers, or other kinds of modifiers of gene action. They may shift from one location to another within the chromosome complement without losing their identity in the process. A further effect, involving a structurally modified chromosome in the presence of which marked changes in chromosome organization and also in gene action take place, is also under investigation.

Kaufmann and his associates have continued their studies on patterns of chromosome organization. They have interpreted electron micrographs of salivary glands of *Drosophila* and of dividing cells of *Tradescantia* as indicating that the patterns of organization of their chromosomes represent a hierarchy of pairs of helically disposed chromonemata. In *Tradescantia* there are at least 64 (and probably 128) subsidiary units in the somatic prophase chromosomes of the staminate-hair cells. It has been concluded, from studies of the effects of various chemicals such as ribonuclease, ethylenediaminetetraacetic acid, and calcium chloride on *Drosophila* and onion roots, that any agent which disrupts the normal metabolism of the cell can lead to instability that is reflected in modification of chromosome form and behavior.

Several years ago a particularly interesting phenomenon was discovered with the electron microscope, involving a curious "blebbing" of the nuclear membrane into the cytoplasm. Gay has now determined the stage of development of the salivary-gland cell in *Drosophila* at which blebbing takes place and has found that it is correlated with the assumption of a new function by the cell. This finding gives strong support to the hypothesis that nuclear-membrane blebbing is a manifestation of a new mechanism for genetic control of cytoplasmic function. The discovery that deoxyribonucleic acid is included within the blebs, and that ribonucleic acid constitutes part of the associated highly differentiated chromosomal material, adds further weight to this hypothesis.

Perhaps the two most dynamic parts of present-day genetic research lie in the realm of physiological genetics, concerned with the organization and mode of function of the chromosome, the whole nucleus, and the cytoplasm from an essentially molecular viewpoint, and in that of population genetics, concerned with the organization and functioning of a whole population from the standpoint of the individuals composing it. Most of the work in genetics within the Carnegie Institution is concentrated in the former field. Some work in the latter, however, is also being carried forward in the Department of Genetics. Thus during the year Moser completed a mathematical analysis of the dynamics of bacterial populations. The theory provides analytical background, among other things, for the calculation of bacterial mutation rates and specific-selection pressures, and of their standard errors.

It is a long step from the study of biological organization at the level of the chromosome to that at the level of the population. An intermediate level is provided by the organization of cells that go to form a many-celled plant or animal. The interest of the Department of Embryology centers at this level, taking the animal embryo as the example *par excellence* of this level of organization in perhaps its most dynamic developmental phase.

The cellular processes occurring during embryonic development, the period in which the fundamental character and specificity of the tissues composing the organism are established, present a series of challenging problems. Whereas in the adult, under normal conditions, the cells produce molecular species essentially similar to those already present, during embryogenesis and under certain other developmental conditions such as regeneration, other types of synthesis must occur. It has been argued that chemical kinetic theory provides several different models for systems of interacting synthetic processes which will change progressively toward one of a number of alternative steady states, the direction depending on the initial conditions. During the year, Ebert has continued to explore the chemodifferentiation of the heart-forming areas of the early chick embryo. Distinct metabolic properties of an organ-forming area have here been demonstrated for the first time. It has been shown that antimycin A acts, in the effective concentration range, as a specific inhibitor of heart formation. Sodium fluoride is known to act similarly. These specific effects have been contrasted with the general inhibition of development by other inhibitors, including amino acid analogues. A most striking finding was also made in connection with the sodium fluoride effect. Embryos cultured in one-hundredth molar sodium fluoride for two to five hours showed marked areas of degeneration corresponding in position (at stage 5 of the Hamburger and Hamilton series) to the localization of cardiac actin and myosin, and to the heart-forming areas as revealed by transplantation techniques. At stages 6, 7, and 8 the position of the degenerating regions reflected the anteromedial movements of the heart-forming cells.

These provocative findings pose a series of further questions, among them: In the region affected by the inhibitor, is the synthesis of the cardiac proteins blocked, or is the effect at the level of morphogenesis, on the fashioning of the contractile elements into their characteristic structure? Is there an alteration in the pattern of differentiation of the sarcosomes? What are the relations between mitochondrial differentiation and the course of muscle fibrinogenesis? Answering these questions will be a challenging task.

An important part of the research effort of the Department during the past year has been directed toward a fuller understanding of the critical period in mammalian development in which the embryo is transported to the uterus, becomes implanted, and begins its uterine career. Böving has carried on interesting studies on the mechanism of the remarkably even spacing of blasto-

cysts along the rabbit uterus, hitherto very imperfectly understood. It was found that the "spacing" was accomplished by the delicate interaction of two progesterone-dependent mechanisms. It also seems probable that blastocyst diameter plays a role in the phenomenon. Greenwald has taken as a point of departure for a similar investigation an analysis of the mechanism of interruption of implantation and maintenance of the rabbit blastocyst by injected estrogen. It has been confirmed that (following earlier preliminary experiments of Csapo) as little as 5 gamma of estradiol daily for three days, commencing on the second day after fertilization, will interrupt pregnancy in the rabbit.

Among the many other lines of investigation under way in the Department, three further ones are of such general interest as to warrant special mention in so brief a summary.

Bishop successfully concluded a series of studies of the nature of the fluid of the rabbit oviduct. His research was directed toward the elucidation of the factors controlling the release of the tubal fluid and toward an analysis of its biochemical composition, with special emphasis on the substrates for the metabolism of sperm. These studies of the tubal fluid, which has been shown to be secreted under the influence of estrogen, have revealed an absence of glucose and fructose but the presence of significant quantities of lactate and phospholipid. The absence of fructose, long regarded by many investigators as the normal physiological substrate for sperm, and the demonstration, through the use of the oxygen microcathode, that the interior of the oviduct presents an aerobic environment, indicate that the older idea that sperm and eggs carry on obligate anaerobic glycolysis is incorrect. In the absence of readily available tubal substrates, the sperm must utilize endogenous phospholipid reserves; this fact is believed to be one of the deciding factors in sperm survival and ability to fertilize eggs.

Bartelmez has made notable progress in his analysis of the vascular changes during the menstrual cycle in the rhesus monkey. The problem is to correlate the changing pattern of the coiled arteries of the uterus with the events of the hormonally controlled reproductive cycle. On the basis of his detailed description of the vasculature of the endometrium (the lining of the uterus), Bartelmez has advanced the suggestion that the rate of blood flow determines the hormonal responsiveness of the various regions of the endometrium. He has also presented evidence that the loss of tissue from the endometrium during menstruation does not involve three-fourths of the membrane, as is usually taught. The increased contortion of the coiled arteries is a striking feature of menstruation, but it appears that only the superficial branches of these arteries are lost during ovulatory menstruation. Bartelmez argues that the reduction in thickness of the endometrium is due largely to a reduction in size of the



component cells in the course of their dedifferentiation, and a diminution in mass of the intercellular ground substance.

These studies by Bartelmez are complemented by the closely related investigations of Ramsey, who, partly in collaboration with Bartelmez and Böving, has confirmed and extended her earlier conclusions concerning the vascular patterns of the placenta. She has shown that maternal blood enters the intervillous space, the terminal area of metabolic exchange between the maternal and fetal blood streams, from small blood vessels, the spiral arterioles, under a head of maternal pressure. It does not "short-cut" into venous exits even when they are contiguous to the arterial entry points along the base of the placenta. In addition, it has been demonstrated clearly that the arterial units are independent: not all arteries contract and relax at the same time. The separation of afferent and efferent streams is effected primarily by pressure differentials in the intervillous space, assisted by features of the morphological structure.

De Feo and Reynolds completed the first phase of a program designed to study the effects of the alkaloid reserpine on the reproductive cycle of the rhesus monkey. The observation, by others, that in rats ovulation may be suppressed when the drug is given in proestrus suggested that reserpine might provide an effective pharmacologic block to ovulation in the monkey, as well as in other primates including man. Although the research must be extended to include a study of possible seasonal variations, the initial results are provocative. Reserpine suppresses menstruation and ovulation when it is administered throughout the reproductive cycle. It also influences the cyclic pattern when it is given at the beginning of the cycle, that is, through the first twelve days: menstruation is suppressed, ovulation does not occur. When administration of the drug is begun later in the cycle, however, no effect on ovulation is observed. These observations are noteworthy not only because of their importance to a better understanding of the relation of the hypothalamus (through which reserpine is said to act) to ovulatory mechanisms, but also because of their implications with respect to the possible adverse effects of reserpine and related drugs, widely and often indiscriminately used as tranquilizing agents.

The Department of Plant Biology, like the Department of Genetics, is concerned with phenomena involved in growth and development at two very different organizational levels: the level of cellular and intracellular organization, and the level of the population. They are represented in the Department of Plant Biology respectively by an intensive study of the mode and mechanisms of photosynthesis, and by studies in experimental taxonomy. In both areas the Department has a long and active tradition.

On the first front, the year has brought some exceedingly interesting and

suggestive advances. A finding which is still qualitative and preliminary, but of great interest, suggests an active participation of carotene in the photochemical action of chloroplasts. When chloroplasts were extracted from a leaf, frozen and dried and fragmented, and treated dry with petroleum ether as a solvent, they were found to have lost all but 10 to 20 per cent of their photochemical activity as indicated by a dye-reduction reaction. When these inactive fragments, as a dry powder, were immersed again in the solvent fraction and the liquid was evaporated, it was found that the photochemical activity had been restored nearly to normal. Activity could also be restored, however, by adding  $\beta$ -carotene from a laboratory supply. This experiment may well open an avenue to removing and substituting carotenoid pigments in the photosynthetic system in such a fashion that their part in photosynthesis, if it exists, can be explored.

A second investigation of great interest concerns the development of a method for measuring the transformation of protochlorophyll to chlorophyll, by spectrophotometric methods, directly in the living leaf. Hitherto, the transformation of protochlorophyll to the much darker green photosynthesizing chlorophyll, which takes place rapidly when living leaves grown in the dark are illuminated, had to be studied either by fluorescence spectroscopy of killed leaves or by subsequent extraction of the pigments themselves. Thanks to a method developed by Shibata, it is now possible to measure the complete absorption spectrum of protochlorophyll and of chlorophyll directly in the living leaf while the transformation is actually taking place. In consequence, a previously unsuspected intermediate form of chlorophyll was discovered. This new intermediate, recognized by its absorption peak at 684 millimicrons, is formed immediately when the dark-grown leaves are illuminated. Furthermore, Shibata found that this intermediate form changes in about ten minutes, without the need for further illumination, into the form absorbing at 671 millimicrons which had previously been considered by some workers to be the first intermediate form of chlorophyll. After about two hours more in dark or light, the normal form of chlorophyll absorbing at 678 millimicrons predominates.

Smith and Kupke have continued their basic work on the purification and characterization of the protochlorophyll-protein complex—the “holochrome”—from dark-grown leaves, which has been reported previously. The ultracentrifugation methods previously described have been supplemented by a procedure employing fractional precipitation with salts. It now seems clear that active protochlorophyll is attached to particles of uniform size, having an estimated molecular weight of about 400,000, largely protein in character, and nearly spherical in shape. Partially purified preparations of these particles may be frozen and dried without losing their ability to transform their protochlorophyll into chlorophyll on illumination. It is truly remarkable that

a transformation of this kind, so fundamental to the whole photosynthetic process in the living plant, can take place in dry particles, when illuminated, as readily as if the particles were in water.

Several investigations in experimental taxonomy which have been under way in the Department for many years are now being brought to a conclusion. In the program involving range grasses, apomictic lines of hybrids of *Poa ampla*  $\times$  *P. arida*, a most promising combination, have been established for the first time. New plantings of selected range-grass hybrids, to permit more precise comparisons of their climatic responses under varying conditions, have been undertaken. A comprehensive monograph has been completed describing the genetic constitution and the patterns of evolution of climatic races of the plant *Potentilla* under various climatic and ecological conditions. Extensive experiments designed to substantiate and elaborate ideas evolved during the past decade are throwing new light on evolutionary mechanisms and species relationships in the yarrow, a common and widely distributed plant. Genetic experiments are in progress with species hybrids of lowland and altitudinal races of the monkey flower, *Mimulus*. Preliminary work with the tiny floating plant *Lemna*, common on the surfaces of ponds and quiet streams over a large part of the land surface of the world, has shown this plant to be of great interest and adaptability for various investigations in "physiological ecology." Investigations of a similar kind are also in progress with *Artemisia*, that widely distributed colonizer of western open lands, the sagebrush.

Reluctantly, the Carnegie Institution has decided to leave the field of Middle American archaeology. In accordance with the decision that its work in this field will be discontinued in about two years, the Department of Archaeology has concentrated its efforts on the completion of its current program of studies. Members of the staff have this year been primarily engaged in the preparation of preliminary reports covering field work, the study of collections, and the analysis of accumulated data. The final results of these studies will appear somewhat later. A full account of their current status will be found in the report of the Department for this year.

### *Losses . . .*

The death of two distinguished retired directors and three retired staff members of the Institution this year has brought a deep sense of loss.

In the Year Book for 1904 it is recorded that an astronomer-staff member of the Institution, Walter S. Adams, took readings at stated hours with a 3¼-inch telescope to determine seeing conditions on Mount Wilson. As one of the small party that went with George Ellery Hale to set up the Mount



Wilson Observatory in California, Walter Sydney Adams witnessed in the forty years of his association with that Observatory the establishment and full development of the greatest center for observational astronomical research in the world. First as Assistant Astronomer at the Mount Wilson Observatory, and then successively as Acting Director (1910-1911), Assistant Director (1913-1923), and Director (1924-1945), Dr. Adams played a leading part in almost every major activity. He had an important role in the planning of the 200-inch Hale Telescope and the building of the new Observatory on Mount Palomar. His death on May 11, 1956, brought to a close a most distinguished career in the field of astronomy.

Dr. Adams' chief enthusiasm and interest centered in solar and stellar spectroscopy. His early studies were concerned with the laws of the rotation of the sun, and in his spectroscopic investigations he made careful measurements of the Doppler displacements at different solar latitudes. His measurements significantly raised the level of accuracy in observations at the time, and they are still of value. In 1914 Dr. Adams was elected an Associate of the Royal Astronomical Society; in 1917 he received its Gold Medal for his studies on the determination of the absolute magnitude of stars, his interpretation of stellar spectra of different types, and the spectroscopic determination of stellar parallaxes. His later investigations on high-dispersion stellar spectra revealed important new data about the nature and motions of interstellar gases.

During World War II Dr. Adams was in charge of all work at the Mount Wilson Observatory under war contracts concerned with optics, optical instruments, aerial photography, and the efficiency of types of bomber forms. He was a consultant to the National Defense Research Committee from 1941 to 1945. He was instrumental, too, in keeping the International Astronomical Union alive during the war, serving as its vice-president from 1935 to 1949.

Dr. Adams' scientific achievements brought him many honors, including election to the National Academy of Sciences in 1917 and awards of the Draper Medal and Bruce Medal in America and the Prix Janssen and the Janssen Medal in France.

Dr. John A. Fleming, internationally known authority on geophysics, died on July 29, 1956, at the age of seventy-nine. He had been associated with the Institution for a half century, joining the staff of the newly established Department of Terrestrial Magnetism in 1904, and becoming Acting Director in 1929 and Director in 1935. From the time of his retirement in 1946 until 1954 he served as Adviser in International Scientific Relations for the Institution.

Dr. Fleming was a man of amazing energy. Under his direction the Department became a leading center of research on the earth's magnetic and

electric fields. An interesting and especially significant line of investigation pursued under his leadership was an estimation of the height of the ionized layers in the atmosphere by means of radio waves. Out of this came the Breit-Tuве pulse technique for sending and recording the return of radio waves, a technique that later became the basis for radar. One of the exciting events that occurred in the Department's laboratory during Dr. Fleming's directorship was the demonstration in January 1939 of nuclear fission, thirty-six hours after the telegram "Barium comes from uranium" came from Copenhagen to Niels Bohr, who was then in Washington attending a conference jointly sponsored by the Carnegie Institution and the George Washington University.

During World War I Dr. Fleming worked on the perfection of a magnetic type of underwater mine that was used extensively in the North Sea area, and developed a special long-period gimbal-ring support for the mounting of a submarine detection device. During World War II, as Director of the Department of Terrestrial Magnetism, he was in charge of all work under Institution war contracts concerned with ordnance devices including the proximity fuze, radio-wave and communications improvement, magnetic compasses and odographs, and ionospheric, hydrographic, and radioactivity studies.

In addition to his scientific work Dr. Fleming found time to edit the *Journal of Terrestrial Magnetism and Atmospheric Electricity* (now the *Journal of Geophysical Research*) from 1932 to 1948, and the *Transactions of the American Geophysical Union* from 1925 to 1947. His duties as general secretary of the Union during the same period included the management of its business affairs.

Many honors came to Dr. Fleming during his lifetime: honorary degrees from the University of Cincinnati and Dartmouth College, election to the National Academy of Sciences in 1938, the William Bowie Medal from the American Geophysical Union in 1941, the Charles Chree medal and prize of the Physical Society of London in 1945, and the Commander's Cross of the Royal Order of Saint Olav of Norway in 1948.

Dr. George Sarton, a Research Associate of the Institution in the history of science from 1918 until his retirement on September 1, 1949, died on March 22, 1956, in Cambridge, Massachusetts. Born in Ghent, Belgium, August 31, 1884, and educated at the University of Ghent, Dr. Sarton went to England at the outbreak of World War I and came to the United States in 1915, where he first taught at the University of Illinois and George Washington University, and then became associated with the Institution. While maintaining his connection with the Institution, he joined the staff of Harvard University in 1920, becoming a full professor in 1940.

In his chosen field of the history of science, Dr. Sarton stood pre-eminent.



As an advocate of what he termed “the New Humanism,” he studied all aspects of culture in their relation to the central theme of his interest—the development of science. His researches traversed the fields of theology, art, linguistics, geography, politics, and the technical arts. His writings were prodigious. Under the aegis of the Institution he wrote a monumental three-volume survey of the sources in the history of science through the fourteenth century, entitled *An Introduction to the History of Science*.

Dr. Sarton founded and for many years edited the journal *Isis*, a review of the history and philosophy of science, and its companion *Osiris*, where substantive studies in the same fields were published. At the time of his death, Dr. Sarton was working on a projected eight-volume *History of Science* from its beginnings to 1900.

Dr. Earl H. Morris died on June 24, 1956. Except for an early seven years with the American Museum of Natural History, his scientific career from 1924 to his retirement on June 30, 1955, was spent entirely with the Division of Historical Research (now the Department of Archaeology) of the Carnegie Institution.

After leading expeditions to Guatemala, New Mexico, and Arizona, and exploring the Aztec ruins in New Mexico, he directed the Institution excavations in Yucatan from 1924 to 1926. His major achievement there was the excavation and restoration of the Temple of the Warriors at Chichen Itza. After 1929, Dr. Morris devoted himself exclusively to research in the origin and development of Southwestern culture, and he became the outstanding authority on Pueblo–Cliff Dweller architecture. His advice and services were frequently requested by the National Park Service in connection with important projects on the Aztec (New Mexico) and Canyon de Chelly (Arizona) National Monuments and the Mesa Verde National Park in Colorado.

Dr. Morris possessed an unusual combination of gifts: aesthetic sense coupled with engineering skill and ingenuity, and a lucid literary style. He was the author of *The Temple of the Warriors* and of numerous papers in scientific journals. His signal contribution to Southwestern and Middle American archaeology earned the Alfred Vincent Kidder Award of the American Anthropological Association for 1953.

Dr. Norman Levi Bowen, who joined the staff of the Geophysical Laboratory of the Institution in 1910 and retired on June 30, 1952, died at his home in Washington on September 11, 1956. During his long association with the Laboratory—broken in 1919 when he served for a year as Professor of Mineralogy at Queen’s University in Kingston, Ontario, and again (1937–1947) when he was Distinguished Service Professor of Petrology at the University of Chicago—he carried on important studies in the phase equilibria of

mineral systems at high temperatures and published the classic monograph *The Evolution of Igneous Rocks* (1928). Dr. Bowen was especially noted for his success in bringing chemical knowledge to bear on the problems of geology through new techniques of investigation in the laboratory and the correlation of laboratory findings with field evidence. His outstanding contributions to science were recognized by election to the National Academy of Sciences in 1935 and to membership in the Royal Society of London in 1949. The highest award of the Geological Society of London, the Wollaston Medal, was presented to him in 1950, and in the same year he received the Roebling Medal of the Mineralogical Society of America. In 1953 he was the recipient of the Hayden Memorial Geological Award from the Academy of Natural Sciences of Philadelphia.

Two staff members and the Director of a department have retired from active service with the Institution.

Dr. Edison Pettit, who came to the Institution from the Yerkes Observatory in 1920, retired as Astronomer of the Mount Wilson and Palomar Observatories, Pasadena, California, on June 30, 1955, after thirty years of service. Dr. Pettit, in collaboration with Dr. Seth Nicholson, was responsible for the development of sensitive vacuum thermocouples for measuring the heat radiation of celestial bodies. By this means he obtained fundamental results that are still in use after almost three decades.

Pettit's long and detailed study of nebulae, starting with a 6-inch and ending with the 100-inch telescope, was concerned especially with T Coronae Borealis and Nova Puppis. During the past ten years Dr. Pettit has used photometric methods in measuring the total brightness and colors of some 563 extragalactic nebulae, with results of importance for observational aspects of the apparent expansion of the universe. The scope of Dr. Pettit's work is attested by his numerous articles in the *Publications of the Astronomical Society of the Pacific* and in the *Astrophysical Journal*.

Dr. Jens Clausen, of the Department of Plant Biology, retired on June 30, 1956. He came to the Department from Denmark in 1931 as an investigator in cytology and genetics, to continue on a broader scale the work of Dr. Harvey Monroe Hall in the field of experimental taxonomy. With the later collaboration of David D. Keck, taxonomist, and William M. Hiesey, physiologist, he conducted a series of long-range field and laboratory experiments on the forces that govern the distribution and evolution of plants under natural conditions. Their detailed findings and general conclusions have been embodied in a series entitled *Experimental Studies on the Nature of Species*, the fourth volume of which is now in press. The series is considered a classic in its field. In 1949 this group of investigators was awarded the Mary

Soper Pope Medal of the Cranbrook Institute of Science for distinguished accomplishment in botanical sciences.

Since 1943 Dr. Clausen has also made important contributions to the study of the genetics of various forage grasses in the course of developing strains suitable for planting under the widely different climatic conditions of the nation's great cattle ranges.

In recognition of his contributions in the field of plant biology, Dr. Clausen was elected in 1951 to membership in the Royal Academy of Sciences and Letters of Denmark, and in 1955 he was elected a foreign member of the Royal Swedish Academy of Sciences. In that year, also, he served as vice-president of the Society for the Study of Evolution, and has this year served as its president.

Dr. George Washington Corner retired on June 30, 1956, after serving nearly sixteen years as Director of the Institution's Department of Embryology in Baltimore, Maryland.

Before coming to the Institution Dr. Corner was an assistant professor of anatomy at the University of California (1915-1919), associate professor at the Johns Hopkins University (1919-1923), professor at the University of Rochester (1923-1940), and guest lecturer at the Royal College of Surgeons and the Universities of London, Manchester, and Edinburgh. On leave from the Institution from 1952 to 1953, he occupied the chair of George Eastman Visiting Professor at the University of Oxford and was an Honorary Fellow of Balliol College.

Under his direction the Department of Embryology continued the programs mapped out by his predecessors, but gave increasing emphasis to the physiological, chemical, and physical problems of embryology and of mammalian reproduction. For more than forty years Dr. Corner has carried forward classic research in the anatomy and physiology of mammalian and especially of primate reproduction. With Willard Allen, he was responsible for the isolation of the hormone now known as progesterone, which led to major developments in the whole field of endocrinology.

In addition to his many scientific contributions, Dr. Corner has written a number of books, including several in the fields of medical history and sex education, addressed to the general public. He has received very many high honors, including among others the degree of Doctor of Science, *honoris causa*, from Oxford University. He is an Honorary Fellow of the Royal Society of Edinburgh and a Foreign Member of the Royal Society of London. He has recently served as vice-president of the National Academy of Sciences and of the American Philosophical Society.

Dr. Corner's retirement from the Institution brings no diminution of his active work. He is now a staff member of the Rockefeller Institute for Medical

Research in New York City, filling two disparate and congenial roles: as historian of the Institute and as active researcher and adviser in the physiology of reproduction.

*. . . and Gains*

A notable addition to the Institution's staff during the year is Dr. James D. Ebert, who succeeded Dr. Corner as Director of the Department of Embryology on January 1, 1956.

Dr. Ebert received his Ph.D. at Johns Hopkins University in 1950, and taught for a year at the Massachusetts Institute of Technology. In 1951 he joined the faculty of Indiana University, and there instituted a program of research and advanced teaching in experimental embryology. His principal work has been directed toward an understanding of the mechanism of synthesis and the interaction of tissue-specific proteins in development. Since 1952 he has combined the established methods of experimental embryology with radiobiological techniques in an investigation of the growth-regulating mechanisms of the chick embryo. Under his leadership the Department will continue to pursue its present researches and to make its resources available to visiting investigators in embryology and related areas. Increasing attention, however, will be paid to a study of the basic mechanisms of differentiation, growth, and morphogenesis in the early embryo.

It gives me great pleasure to announce that before his retirement as President of the Institution Dr. Vannevar Bush was informed that he had been made an Officer of the Legion of Honor as a token of the high esteem in which France holds his outstanding achievements in the field of scientific research, by decree of the President. In October 1956 he was made an Honorary Fellow of the American College of Surgeons.

The following honors have been received by staff members during the year:

Dr. Ira S. Bowen, Director of the Mount Wilson and Palomar Observatories, was elected an Honorary Fellow of the Indian Academy of Sciences when it met in Hyderabad. Dr. J. Eric S. Thompson, staff member of the Department of Archaeology, was awarded the Viking Medal in archaeology. Dr. Robert K. Burns, staff member of the Department of Embryology, was awarded a John Guggenheim Memorial Foundation Fellowship for travel in Europe to observe such work in progress in a number of laboratories in the field of experimental control of sex differentiation.

CARYL P. HASKINS



# REPORTS OF DEPARTMENTS AND SPECIAL STUDIES

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MOUNT WILSON AND PALOMAR  
OBSERVATORIES

JOINT COMMITTEE ON IMAGE TUBES  
FOR TELESCOPES

DEPARTMENT OF TERRESTRIAL  
MAGNETISM

GEOPHYSICAL LABORATORY

DEPARTMENT OF PLANT BIOLOGY

DEPARTMENT OF EMBRYOLOGY

DEPARTMENT OF GENETICS

DEPARTMENT OF ARCHAEOLOGY



# MOUNT WILSON AND PALOMAR OBSERVATORIES

OPERATED BY THE CARNEGIE INSTITUTION OF WASHINGTON  
AND THE CALIFORNIA INSTITUTE OF TECHNOLOGY

*Pasadena, California*

IRA S. BOWEN, *Director*

## OBSERVATORY COMMITTEE

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One of the most important fields of investigation of present-day astronomy is stellar evolution, which seeks answers to such questions as: How are stars formed? What is their lifetime? How do their various observable properties such as absolute brightness, surface temperature, and chemical composition change during this lifetime? The first key to answering them came during the past two decades from studies of nuclear physics. These studies showed that a hydrogen nucleus or proton can react either directly with other protons or through the agency of heavier nuclei acting as catalysts to form helium with the release of enormous amounts of energy. They also gave information about the rates of such reactions as a function of temperature and density. These results, combined with earlier theoretical studies of temperature and pressure conditions in the interior of the stars, provided conclusive evidence that the formation of helium from hydrogen in the stellar core is the chief source of the enormous amount of energy which the stars radiate.

According to present concepts, stars are originally formed by the condensation of clouds of material, chiefly hydrogen. As the cloud contracts into a star its core heats to the point that transformation of hydrogen into helium occurs. Soon a state is reached in which the formation of energy just balances the losses by radiation. In this initial steady state the brightness of the star and its surface temperature vary rapidly with the mass that has condensed to form

the star. Thus a star with a mass 12 times that of the sun gives off 1000 times the light of the sun and has a surface temperature of  $23,000^{\circ}\text{C}$ , or 4 times that of the sun. On the other hand, a mass  $\frac{1}{7}$  that of the sun radiates energy at only  $1/10,000$  the rate of the sun and has a surface temperature of  $3000^{\circ}\text{C}$ . Stars that are functioning in this initial equilibrium state and with this relationship between mass, luminosity, and surface temperature are called main-sequence stars.

As time goes on, however, the hydrogen fuel is consumed, and eventually the composition of the star is so changed that the initial equilibrium conditions no longer hold. When this occurs, theory predicts that the star slowly expands and the surface cools, the star appearing redder. At the same time the total radiation of the star increases. As the hydrogen is still further exhausted, the process reverses; the star contracts, and becomes hotter but fainter. At one or more stages in this evolution the star becomes unstable and pulsates rapidly with a corresponding oscillation in its brightness. Eventually as the fuel is completely exhausted, the star stops radiating and can no longer be observed. It is obvious that the bright stars which are burning their hydrogen much more rapidly than the fainter stars will go through this cycle faster than their fainter neighbors.

This picture at once provides an explanation of Baade's observation of the different types of stellar populations. Thus in the

spiral arms of our own Galaxy or of the Andromeda galaxy a substantial fraction of the mass still exists in the form of clouds of gas and dust. Since these clouds are still condensing and forming stars, many young stars are still present. The most conspicuous features of this stellar Population I are the very massive, exceedingly bright, and very hot blue stars.

In the central region of these galaxies no dust or gas clouds are present, and consequently all stars must have been formed billions of years ago; all the original very bright blue stars have used up their fuel and disappeared. The brightest stars of this stellar Population II are therefore the stars of initial intermediate brightness that have just reached the stage in their evolution at which they expand and cool off but at the same time become brighter. The most conspicuous stars in this population are therefore cool and red.

In this broad outline of present theories many parts must be refined and the details filled in. Further laboratory measurements, astronomical observations, and theoretical studies will be required to complete the picture. The close co-operation between the Observatories and the Physics Department of the California Institute of Technology has made possible an effective attack on all sides of the problem. Thus Dr. William A. Fowler and his collaborators at the Kellogg Radiation Laboratory have greatly improved the measurements of the reaction rates for the various processes by which hydrogen is transformed into helium. More recently, in an effort to explain the present chemical composition of the stars, this group has studied the reactions by which the heavier elements may be formed in the stellar cores.

From the observational side it is important to determine the changes in the brightness and surface temperature of a star as its hydrogen fuel is consumed. Unfortunately, we cannot observe a given star throughout its life history, which often extends over many billions of years. We can, however, observe a group of stars of the same age

which, because of a wide distribution in mass and therefore in rates of evolution, have progressed for different relative distances along their evolutionary paths. Sample groups of stars of the same age are provided by the various globular and galactic clusters of stars. Since the globular clusters contain no dust or gas, all their stars presumably date back a few billion years to the period of initial star formation. So-called open clusters that have recently emerged from the clouds of dust and gas of the Milky Way provide examples of younger stars condensed from these clouds.

A substantial number of investigations have recently been carried through or are still in progress at the Observatories, on the relation between the absolute brightness and the surface temperature as indicated by color index, for the stars of a number of clusters. A comparison of the shape of, and the distribution of stars along, the color-magnitude diagram thus obtained with the color-magnitude diagram of the main-sequence stars yields information on the manner and rate at which the properties of stars of various masses change with age. Extensive spectroscopic investigations are in progress both on main-sequence stars and on stars in the globular clusters to determine their chemical compositions for comparison with the predictions made from laboratory studies of reaction rates. The combined result of all these laboratory, theoretical, and observational studies has led to a greatly increased understanding of stars, the source of the energy they radiate, and their evolutionary history.

The present report year has seen the completion of the National Geographic Society-Palomar Observatory Sky Survey. This survey, which was very generously financed by the National Geographic Society, has occupied practically the whole observing time of the 48-inch schmidt camera for the seven years beginning in July 1949. The whole sky accessible from Palomar Mountain, i.e., north of declination  $-27^\circ$ , has been photographed in both red and blue light, by Dr. Albert G. Wilson,



Abell, and Harrington. This project required 879  $14 \times 14$  inch plates in each color. The blue plates reach a limiting magnitude of 21.1 and the red plates 20.0, corresponding to a penetration approximately 3 times farther into space than has been achieved on any previous survey of the whole sky. Objects have therefore been recorded throughout a volume of space about 25 times as large as in previous atlases.

In order to maintain a uniformly high standard for all fields a substantial fraction of the pairs of plates taken had to be rejected because of poor seeing conditions, errors in guiding, poor focus, emulsion defects, and the like. A complete year-by-year breakdown since the Survey began in July 1949 is given in table 1. In a few cases more than one pair of acceptable plates have been obtained for individual fields. The last column of the table, therefore, totals more than 879, the number of fields photographed.

Ninety-eight copies of the atlas in the form of 1758 photographic prints have been ordered, 42 by institutions in the United States and 56 by foreign observatories. At the end of the report year 600 sheets of the atlas had been printed by the Graphic Arts Facilities of the California Institute and shipped to the subscribers.

Because of the enormous mass of material on the atlas plates many decades will be required to exploit them fully. Already

a rapid qualitative survey of the plates has noted many new objects, including 4 unusual asteroids whose orbits pass close to that of the earth, 11 comets, 13 globular clusters, and 82 planetary nebulae. Four new dwarf members of our local group of galaxies have also been discovered. The survey has been especially effective in re-

TABLE 1

Date	Total pairs taken	Acceptable pairs
July 19, 1949 to June 30, 1950...	161	81
July 1, 1950	1951... 94	37
1951	1952... 270	100
1952	1953... 230	120
1953	1954... 356	225
1954	1955... 331	240
1955	1956... 164	91
Totals .....	1606	894

cording faint galaxies and clusters of galaxies. In a special search of the plates Abell has found and listed the position of about 2700 very rich clusters of galaxies, thereby increasing manyfold the number of known objects of this type. The statistics of the size and distribution of these clusters is of great importance, since they are the largest known structures in the universe and consequently can be observed through the greatest volume of space.

## OBSERVING CONDITIONS

The precipitation on Mount Wilson was 28.15 inches for the year 1955-1956. Of the past nine years, eight—including the last four—have had below-normal rainfall, the nine-year average being less than three-fourths of the mean of the 52-year period

since records were started. Solar observations were made on 337 days between July 1, 1955 and June 30, 1956. During this same period observations were made with the 60-inch on 305 nights and with the 100-inch on 317 nights.

## SOLAR RESEARCH

## SOLAR PHOTOGRAPHY

Solar observations were made by Cragg, Hickox, Nicholson, and Richardson. The numbers of photographs of various kinds taken between July 1, 1955 and June 30, 1956 were as follows:

Direct photographs .....	674
H $\alpha$ spectroheliograms, 60-foot focus ...	672
H $\alpha$ spectroheliograms, 18-foot focus ...	1,011
K $2$ spectroheliograms, 18-foot focus ...	969
K $2$ spectroheliograms, 7-foot focus ...	68,000
K prominences, 18-foot focus .....	975

## SUNSPOT ACTIVITY

The magnetic classification and study of sunspots and related phenomena have been continued by Nicholson and Cragg. Co-operative programs have been carried out with the U. S. Naval Observatory, the University of Michigan, the Observatory of Kodaikanal, the Meudon Observatory, the Central Radio Propagation Laboratory of the National Bureau of Standards, and the Royal Greenwich Observatory. During the calendar year 1955, solar observations were made at Mount Wilson on 333 days, on 38 of which the sun was without spots. The total number of spot groups observed in 1955 was 208, compared with 46 in 1954, and 93 in 1953. The last group of the old cycle appeared on November 24, 1955. The northern hemisphere was the more active, having 127 groups while the southern had 81. Solar activity has increased since the minimum in 1954 at a rate slightly greater than at the same phase in the previous cycle. In the first half of 1956 it exceeded that at the maximum of 1928, and approached closely that of the maximum in 1937.

The monthly means of the number of groups observed daily for the past two and one-half years are shown in table 2.

Twenty-two flares of intensity 1, seven of intensity 2, and none of intensity 3 were recorded in 1705 hours of observing. The average number of flares per 100 hours of observing was 1.7 in 1955 and 0.2 in 1954.

TABLE 2

MONTH	DAILY NUMBER OF SUNSPOT GROUPS		
	1954	1955	1956
January .....	0.0	2.2	6.4
February .....	0.1	2.2	9.3
March .....	0.5	0.6	10.6
April .....	0.3	1.4	10.8
May .....	0.1	2.3	9.9
June .....	0.0	2.4	9.3
July .....	0.6	3.2	...
August .....	0.8	3.3	...
September .....	0.3	4.9	...
October .....	1.4	6.1	...
November .....	0.9	7.3	...
December .....	0.7	6.9	...
Yearly mean .....	0.5	3.6	...

## MAGNETIC POLARITIES

Magnetic polarities in each spot group have, if possible, been observed at least once. The classification of groups observed between July 1, 1955 and June 30, 1956 is indicated in table 3. "Regular" groups in the northern hemisphere are those in which the preceding members have N (north-seeking) polarity; in the southern hemisphere the polarities are reversed.

TABLE 3

Hemi- sphere	Regu- lar	Irregu- lar	Unclas- sified
North .....	189	2	55
South .....	137	1	42
Whole sun .....	326	3	97

## STUDIES OF GEOMAGNETIC ACTIVITY

The diurnal variation of geomagnetic activity on disturbed days has been studied by Nicholson and Dr. Oliver P. Wulf, of the U. S. Weather Bureau, using the eight 3-hour-range indices (K) for the five selected disturbed days in each month for the nine years 1940-1948 from six low-

latitude stations. Both a local-time component and a Universal-Time component were found as in previous studies of the mean variation for all days. The amplitude of the Universal-Time component is relatively greater than that of the local-time component when only disturbed days are used.

#### SOLAR MAGNETIC FIELDS

Regular daily observations have been continued by H. D. Babcock with the original solar magnetograph at the Hale Solar Laboratory in Pasadena. Concurrently with the growth of the new sunspot cycle, the magnetic records have shown many strong bipolar magnetic (BM) regions, with a general increase not only in number but also in total flux and field intensity. Some of the BM regions, however, are of relatively great extent and low intensity, and many fail to show sunspots. A noteworthy feature has been the rather sharp lower boundary in heliographic latitude for this activity, with scarcely any fields as strong as 1 gauss near the solar equator. Many of the BM regions have extended poleward to latitudes near  $60^\circ$  and higher. The association of these BM regions with hydrogen filaments, or prominences, as shown on the Mount Wilson spectroheliograms, has been studied. There is confirmation of the earlier finding that sharp filaments sometimes divide BM regions into areas of opposite magnetic polarity; other filaments, generally more diffuse, form boundaries between distinct BM regions. Further studies of total magnetic flux and flux distribution within magnetic regions and in the northern and southern solar hemispheres have been made, and it is apparent that more refined measurements of this kind would be useful and informative.

The installation in the 150-foot tower of the second magnetograph which was described last year has been completed, and a number of records have been obtained with it. The expected improvement

in performance over that of the first instrument in Pasadena has been satisfactorily realized; it results from the use of an image slicer to admit about six times as much light to the spectrograph, from the use of two sensitive spectrum lines instead of one, and from the generally better atmospheric conditions. The signal-to-noise ratio is so much improved that it is possible to obtain equivalent records in a much shorter time or, alternatively, to record weaker fields with improved heliometric resolution.

One result obtained by H. W. Babcock with the second magnetograph on Mount Wilson has been the confirmation of the existence, polarity, and order of magnitude of intensity of the poloidal or "general" magnetic field of the sun in high heliographic latitudes; this field has been consistently observed with the first magnetograph in Pasadena for the past four years.

The interpretation of the small Zeeman displacements measured by the magnetograph has been challenged by certain theoreticians on the ground that a weak general field might be undetectable in the presence of stronger turbulent fields in the solar granules, provided that a systematic coupling, asymmetric in magnetic polarity, prevails between the magnetic field and the line intensity. But such an effect would produce a bias underlying all the measured weak fields, and a bias of this sort is definitely excluded by the observations. The absence of any indicated field over large areas of the quiet sun, and the consistent behavior of BM regions, both strong and weak, as regards magnetic polarity, appear to dispose of the objections to the interpretation of small Zeeman effects in terms of magnetic fields. The small-scale hydro-magnetic effects associated with granules are no doubt of much interest in themselves, but they are too minute to be resolved by existing apparatus. Only the average field over relatively large areas can be observed.



## SOLAR SPECTRUM

The profiles of 70 atomic lines in the spectrum of the solar disk center have been photoelectrically measured by Rogerson. The observed profiles, corrected for the smoothing effects of the spectrograph and scanning slit, were subjected to an exploratory analysis. The analysis suggested a turbulent velocity of 1.41 km/sec which appeared to be constant throughout

the absorbing layers of the solar atmosphere. Of interest also is the indication that the line-damping constant is little dependent on parity but strongly dependent on excitation potential. The ratio  $\gamma/\gamma_{\text{cl}}$  varies from around 20 for zero-volt lines to around 300 for 5-volt lines.

An attempt is being made to obtain photoelectric profiles for lines at varying limb distances.

## PLANETS AND COMETS

The most recent spectroscopic measures published on the rotation of Venus appear to be those made by V. M. Slipher at Lowell Observatory in 1903. Similar measures made by C. E. St. John and Nicholson about 1922 were never published.

Richardson has attacked the problem anew, taking advantage of several important improvements now available in the method of observation. The spectra were taken at the Snow telescope, which, although designed for solar work, can readily be adapted for a bright object such as Venus. Moreover, it can be used every night.

A grating was installed which gives a dispersion of 0.8 Å/mm, about 14 times the scale of Slipher's plates. Emulsions sensitive to the red instead of blue can now be used, and give considerably larger Doppler displacements. By using telluric lines instead of iron arc lines as standards, absorption lines can be measured against absorption lines.

A good spectrum could be obtained in 90 minutes. Fifteen plates were taken when Venus was near eastern elongation in the spring of 1956; more will be taken when the planet comes to western elongation in September 1956.

Ultraviolet images of Venus obtained in March 1956 furnish a striking example of the rapid changes of continental dimensions in the atmosphere of the planet. On March 3 there was a well defined funnel-shaped band across the center of the disk, 4700 miles wide at the terminator and

tapering to 1600 miles at the limb. On March 4 the shape of the band was about the same, but the interior had a mottled appearance. On March 5 the disk was blank. The appearance of the band on March 3 and 4 was so nearly the same as to leave no doubt that the same region of the planet faced the earth. The period of rotation, therefore, would appear to be very slow or else almost exactly 24 hours.

Visual observations of Mars and photographs in blue and yellow were made by Richardson at the 60-inch on June 3, during a period of excellent seeing. When seen visually the deserts in the southern hemisphere appeared covered by a pattern of irregular veins of the same color as the dark maria (green through a yellow filter; distinctly blue-green when viewed directly). The pattern did not resemble that of the fine lines with which the canals are usually depicted. Some of the pattern can be discerned on the best of the yellow images. Although Mars was then 74,000,000 miles away, the season was a month after spring in the southern hemisphere, when experience shows that the "canals" should be most conspicuous.

About 500 blue and deep-red images of Jupiter were taken by Richardson with the 60-inch from February 5 to June 4, 1956, using a camera and enlarging lens designed at the Griffith Observatory. The images range from 14 to 19 mm in diameter. These observations were made in co-operation with the Department of Terrestrial Magnetism, in connection with the

radio bursts on 22 Mc/sec discovered by the Department early in 1955.

C. A. Shain in Australia from prediscovery observations has tentatively identified the optical source as a bright oval marking in the South Temperate Belt, which in November 1951 had a longitude of  $355^\circ$ . It was then following the Red Spot by  $100^\circ$ , with a period 30 seconds less than that of the Red Spot.

On March 5, 1956, about 5<sup>h</sup> UT, radio bursts were recorded while observations of Jupiter were in progress. The Red Spot was near the central meridian of the planet at this time, but the most likely source would appear to have been a bright cloud which partly obscured the preceding half of the Red Spot at this time. Its appearance was similar to that of the marking identified by Shain. The photographs of February 5 show this marking over the following portion of the Red Spot. During this interval its period was 34 seconds less than that of the Red Spot, practically the same as that of Shain's marking.

On the *blue* images the Red Spot appears as an *absorption* marking. The bright spot has about the same intensity as the Equatorial Belt. On the *red* images the Red Spot appears as a *bright* marking only slightly less intense than the Equatorial

Belt. The bright marking is slightly fainter than the Red Spot.

Another center of radio disturbance at  $200^\circ$  might be attributed to an oval bright marking at about this longitude following the South Tropical Disturbance.

A third radio disturbance was suspected at about  $40^\circ$ . On the red images there is a small bright marking near this longitude along the southern edge of the North Equatorial Belt. There is no trace of this marking on the blue images.

There is a well defined bright marking on both blue and red images at  $120^\circ$  in the South Temperate Belt similar to the one near the Red Spot. No radio disturbance seems to have been reported from this region.

A series of plates of Comets Baade (1954h) and Haro-Chavira (1954k) was obtained by Osterbrock, with help from Abell and Dr. L. Plaut. These plates are to be used to compare the direction of the comet tail with the antisun direction. According to the ideas of L. Biermann, there may be appreciable deviations of the tails from this direction if the mechanism of tail formation is corpuscular radiation. Preliminary reductions of some of the plates of Comet Baade show such deviations quite strongly.

## STELLAR SPECTROSCOPY AND PHOTOMETRY

Spectroscopic observations have occupied approximately half the time of the major telescopes, 620 spectrograms having been taken during the report year with the 200-inch telescope, 1000 with the 100-inch, and 1700 with the 60-inch.

### VARIABLE STARS

The irregular, explosive variable star AE Aquarii has been observed spectroscopically by Deutsch at the 100-inch telescope, simultaneously with photoelectric observations by Dr. Merle F. Walker at the 60-inch. Spectrograms have been obtained when the star was at normal brightness, and during three explosions that

lasted an hour or more and that reached peak amplitudes in the ultraviolet of more than 2 magnitudes above normal brightness. These spectrograms indicate the emergence of a strong ultraviolet continuum during explosions. To a first approximation, the Balmer emission lines, including H $\alpha$ , and the Balmer emission continuum are unaffected during an explosion. The ultraviolet continuum may possibly be synchrotron radiation; polarization effects will be looked for in the near future.

Coudé spectrograms have been obtained by Deutsch during the chromospheric eclipse of VV Cephei. It appears that



some chromospheric lines of Sc II and Ti II were present in the far ultraviolet more than four years before the beginning of the photometric eclipse in the summer of 1956. The chromospheric lines differ in structure from one element to another, the lines of Ti II being systematically the most complex.

Spectrographic observations of SS Cygni at minimum light were continued by A. H. Joy. With difficulty, faint absorption lines of the dG5 star and the emission lines, 20Å in width, of the sdB component were measured for velocity. Approximate binary elements with a period of 0.276 day were obtained. The hot star, which is about the size of Jupiter, seems to be surrounded by an emission envelope or streamers, in rapid motion. The masses are less than one-half that of the sun, and nearly equal.

The 1954 simultaneous spectrographic and photometric observations by O. C. Wilson and Walker of SX Phoenicis and CC Andromedae have been studied. The most noteworthy results are: (1) For SX Phoenicis ( $P \sim 80^m$ ) the approximate quarter-period phase lag between maximum brightness and maximum size is present just as for cepheids. (2) Although SX Phoenicis shows wide variations in light-amplitude, the corresponding variation in velocity-amplitude is not certain. (3) CC Andromedae turns out to be probably a star of the  $\delta$  Scuti class.

William G. Tifft has continued the program on three-color photoelectric photometry of short-period cluster-type variables of Bailey class c; spectra have also been obtained.

Sandage completed a theoretical study on the masses of the RR Lyrae stars. Results, reported two years ago, of the two-color photometry of selected RR Lyrae type variables in M 3 gave a mass of 0.70 solar mass if the normal relation between color index and effective temperature is used. This mass is a factor 2 too low as compared with the predictions of evolutionary theory. It was shown this year

that the normal relation between color and temperature does not apply to the RR Lyrae stars because of the change in the wavelength distribution of the emergent flux caused by low surface gravity. When the gravity effect is taken into account, a mass of 1.25 solar masses is obtained, a value which is in agreement with theory.

The shell of Nova Puppis 1942 was photographed with the 200-inch by Zwicky; it was found to have a radius of  $\mu = 2.78''$  in red light. Originally the velocity of expansion was 1600 km/sec (and 2400 km/sec). With the measured  $\mu$  and (original) expansion velocity of 1600 km/sec the distance becomes 1600 parsecs, and the nova at maximum would have had an absolute visual magnitude about  $-10.5$ . Since an obscuration of at least 0.5 magnitude must be assumed, Nova Puppis had the highest absolute brightness of any common nova known so far. Its magnitude as well as the character of its light-curve indicate, in fact, that Nova Puppis might have to be classed as the first intermediary between common novae and supernovae.

In September 1955 Zwicky discovered the first known common nova in NGC 205 (companion of the Andromeda galaxy). It was located at  $6'8$  north of the nucleus of NGC 205, and at maximum was probably somewhat brighter than  $m_p = 16.0$ .

Indications have been obtained by Zwicky that some parts of the shell of Nova Persei of 1901 are polarized.

During the preparations for an extended supernova search to be carried out in cooperation with the Lick, Lowell, Steward, and Berne (Switzerland) observatories, H. S. Gates found a supernova  $m_p \sim 11.0$  in NGC 3992. A possible supernova of November 1955 also noted by Gates in NGC 3294 is now being checked. Zwicky found a supernova  $m_p$  (max) = 15.8 in a peculiar galaxy [R.A.  $1^h 5^m 0$ , Decl.  $-13^\circ 29'5$  (1950)], presumably a member of the A cluster at a distance (old scale) of about 100 million light-years. This is the most distant supernova yet found.

P. W. Merrill and Wilson have made a detailed study of the complex lines of the remarkable spectrum of the supergiant M-type star RW Cephei. Certain features resemble those in the spectrum of  $\alpha$  Orionis but are more extreme.

Sanford has completed measures of the radial velocities of T Monocerotis, SV Vulpeculae, and the peculiar bright-line star HD 31293.

#### GLOBULAR AND GALACTIC CLUSTERS

Sandage, in collaboration with Dr. Harold L. Johnson, of the Lowell Observatory, has continued the investigation of color-magnitude diagrams of star clusters and their relation to stellar evolution. The color-magnitude diagrams for the galactic cluster M 11 and photometry in three colors in the globular cluster M 3 have been completed. M 11 is a populous open cluster situated between the observer and the rich Scutum star cloud. This cluster is important for tracing the systematics of evolutionary paths of stars of all masses in the  $M_{bol}$ ,  $\log T_e$  plane, since M 11 is intermediate in age between the Pleiades and the Hyades. The cluster has no main sequence brighter than  $M_v = -1.0$ . There are many yellow giants ranging from  $M_v = -0.8$  to  $M_v = -1.7$ . This result is significant, for it suggests that giants can fill the entire region from  $M_v = +2.0$  to  $M_v = -8.0$  as a result of the evolution of stars of all ages from the main sequence.

The apparent concentration of giants in the general field at  $M_v = 0.0$  has been shown by Sandage to be a natural result of the systematics of the evolutionary tracks for stars of 1.5 to 3.0 solar masses. This apparent sequence of giants does not require an increase in the rate of formation of stars some  $10^9$  years ago (the age of the Hyades giants at  $M_v = +0.5$ ), but is rather a consequence of the details of the individual evolutionary tracks. All stars whose masses range from 1.5 to 3.0 solar masses are mapped from the main sequence into a narrow sequence in the giant region at about  $M_v = +0.5$ .

Three-color photometry has been completed in the globular cluster M 3 by Johnson and Sandage. The photoelectric standards, which were obtained by Johnson using the 82-inch telescope of the McDonald Observatory, reach  $V = 21.9$ , and embrace the entire intensity range covered by the photographic material. Measurements on new photographic plates taken in three colors with the 100- and 200-inch telescopes gave a revised color-magnitude diagram and also provided information on the energy-distribution curves of these globular-cluster stars. These new results show that the main sequence of M 3 does not lie below the sequence of the near-by stars as was found in Baum's measurements of M 13. The M 3 sequence is brighter than the near-by star sequence by about 0.2 magnitude if the RR Lyrae stars are assumed to be at  $M_v = 0.00$ . These results indicate that a difference exists between the globular clusters M 3 and M 13 which, if real, is extremely important. More measurements at these very faint light-levels are obviously needed. Until the results of the various observers agree, the problem cannot be considered solved. All stars in M 3 show an ultraviolet excess ranging from  $\Delta(U-B) = 0.20$  mag. for the giants to  $\Delta(U-B) = 0.55$  mag. for the main-sequence stars. This difference in the energy-distribution curves between the globular-cluster stars and stars of Population I makes any comparison of sequences between the two populations dubious at this time.

As was stated in the annual report for 1953-1954, photoelectric photon counts in the globular cluster M 13 revealed that its main sequence lies about 2 magnitudes below the "normal" main sequence defined by stars in the solar neighborhood. Photon-count observations by Baum at Palomar have since extended this type of measurement to M 3, M 92, and M 15. The investigation of M 15 is being carried out jointly with Osterbrock. Photon-count observations during the report year included five faint stars in M 92 and seven in M 15.

The results thus far in hand indicate that the main sequences of the four clusters are not alike and that they scatter within a 2-magnitude band bounded at one extreme by the main sequence for the solar neighborhood and at the other by that for M 13.

Photometric results by Walker reported in 1953-1954 showed that the more massive members of the young cluster NGC 2264 have already settled down to the main sequence in the O and B regions, while the less massive members, which presumably evolve more slowly, have not yet reached the main sequence. In an effort to extend the investigation to fainter magnitudes where the "least evolved" members lie, observations of 26 stars were obtained by Baum with the photoelectric photon counter on the 200-inch telescope. Photographic plates were also obtained in blue and yellow, so that other stars in the cluster can be measured with reference to the photoelectric standards by photographic interpolation. This material indicates that the faintest members of the cluster are extremely red, that faint blue stars are virtually absent, and that a further extension of the observation will be desirable for completing the faint end of the color-magnitude diagram.

G. Wallerstein has studied the velocity curves and spectral variations (for comparison with W Virginis) of two Population II cepheids in the globular cluster M 5. In addition, he is making comparisons with classic cepheids.

A 160 A/mm plate of one of the bluest stars known in M 13 (Halton C. Arp and Harold L. Johnson No. 110,  $V=17.54$ ,  $B-V=-0.40$ ) has been obtained by Münch with the Palomar prime-focus spectrograph. It shows relatively weak and sharp lines of He I at  $\lambda\lambda 4471$  and  $4026$  and strong hydrogen lines, quite inconsistent with the  $B-V$  color of the star. The large Balmer absorption revealed by the line spectrum and by the ultraviolet "deficiency" reported by Arp and Johnson can be understood only if it takes place in a shell of relatively low density and temper-

ature, surrounding a high-temperature dense core which gives rise to a very blue continuum.

One or more spectra of red giants have now been obtained in each of sixteen globular clusters by Deutsch. A provisional list of the clusters in which the red giants show the weakest metallic lines is the following: NGC 5053, 5466, 5897, 6341 (M 92), 7078 (M 15). Coudé spectrograms of two red giants in NGC 7789, which had been reported as a globular cluster, show the characteristics of normal Population I giants. Spectrograms have been obtained for classification of the brightest stars in M 11 in support of a photometric investigation by Sandage and H. D. Wahlquist.

Spectra of K-type giants in galactic clusters have been obtained by E. M. Burbidge and G. R. Burbidge with the 4-inch camera of the X-spectrograph of the 60-inch telescope. Standard stars covering the same range of spectral type and luminosity from W. W. Morgan's lists have also been photographed, as well as stars from Nancy B. Roman's list of classified spectra of the bright stars of types G-K showing ranges of the peculiarities (weak CN, "4150," weak-lined) listed by her. Stars in the following clusters have been photographed: NGC 752, Hyades, Praesepe, M 67, M 11, NGC 7789. NGC 7789 was thought by V. C. Reddish to be a globular cluster, but Deutsch showed that the spectrum of a member of its giant branch was of Population I type. Plates taken by Sandage have been measured, and a preliminary color-magnitude diagram has been plotted, which confirms that the cluster is a relatively old galactic cluster, not a globular one, and shows that it has an incipient Hertzsprung gap. More observations are being obtained by Sandage in order to determine the color-magnitude diagram accurately.

The clusters listed above break away from the main sequence at different points, indicating different ages; their giants have different masses and, in the case of M 11, different luminosities. By comparison of



the spectra with standard stars it is hoped to classify them accurately for luminosity and hence to look for differences in surface gravity. An attempt will also be made to place the stars within Miss Roman's framework of classification according to the small peculiarities listed above.

#### WHITE DWARFS AND SUBDWARFS

Greenstein has continued his program on white dwarfs. The spectrophotometry of lines has been essentially completed and the measurements have been carried out by Mrs. Matthews. Profiles will be available for a wide range of color and luminosity; most profiles are based on three or more 200-inch coude plates, at 38 Å/mm. A very wide range of hydrogen-line strengths has been found; in a class of "normal" white dwarfs, the lines strengthen monotonically with increased  $B-V$  color. However, many white dwarfs with peculiar spectra exist, notably weak-line objects, the He I white dwarfs, and the  $\lambda 4135$  (unidentified band) objects. Near  $B-V = +0.20$  there is an abrupt sharpening and decrease of hydrogen-line strengths, and Ca II appears. The Ca II lines are not monotonic functions of color, being nearly absent in Wolf 489, and enormously strong for the color in Ross 640. The latter shows a strong Mg I blend at  $\lambda 3835$ , so far visible only in this star. The Ca II lines are sometimes seen in very hot objects, e.g. HZ 19, which has both Ca II and He I. It seems very probable that the white dwarfs differ appreciably in composition, and some may be composite.

The prime-focus spectrograph has been used at Palomar to provide spectra at 180 Å/mm for the fainter objects. New white dwarfs include L770-3, L970-30, L1363-3, and HZ 29. Some extraordinary spectra have been obtained of very blue stars which may be incipient white dwarfs or hot subdwarfs.

During the search of interstellar lines in high-galactic-latitude objects, the star BD +25°4655 was found by Münch to be a helium-rich object, like HZ 44 and

HD 127493. The new O-type subdwarf has slightly lower surface temperature and is brighter ( $m_{pg} = 9.0$ ) than those previously found. Its spectrum, under study on several 10 Å/mm Mount Wilson plates, is extremely rich in faint sharp lines, those of N II, N III, and Ne II being especially conspicuous. The complete absence of lines of oxygen and carbon, in any stage of ionization, suggests that the surface material of this kind of star has undergone nuclear processes which transformed the carbon into nitrogen and the oxygen into neon.

J. Feige has carried out a survey for faint blue stars on the 48-inch schmidt plates. Sixty-five blue stars brighter than  $m 14.5$  were found in fields centered on the north galactic pole, and 17 additional objects in the zone on the northward side of the south galactic pole. One star was found for approximately 25 square degrees. Some spectra and photoelectric colors were obtained. A mixture similar to the Humason-Zwicky stars was found: hot subdwarfs, white dwarfs, horizontal-branch stars, and possibly runaway Population I objects.

#### CHEMICAL COMPOSITION OF STELLAR ATMOSPHERES

A survey of the present state of knowledge of the abundances of the elements has been carried out by Greenstein. Several studies have been made of the abundance differences among normal and peculiar stars, as connected with current theories of the origin of the elements inside the stars. If the apparent deficiency of metals in the extreme F-type subdwarfs is real, it seems plausible that a very considerable time interval elapsed between the formation of the oldest known stars and the sun.

Spectra of the hydrogen-poor and normal Ro stars have been obtained by Greenstein for study of any differences that may exist in their metallic-line spectra. Equivalent widths of about 250 lines in the spectrum of the S star R Andromedae have been measured for comparison of

abundances in this star with those in a normal M giant,  $\mu$  Ursae Majoris. Plates have been taken for a study of the abundance of lithium in M dwarfs.

The Ba II stars have long been known to show enhancement in spectral lines of the ionized elements occurring around the nuclear magic numbers. On the hypothesis that these stars are related to the S stars, and that heavy elements have been synthesized in their interiors by neutron capture on a time scale of the order of  $10^5$  years and mixed to the surface, an attempt is being made by E. M. Burbidge and G. R. Burbidge to calculate the abundances of as many heavy elements as possible. These abundances will be compared with theoretically predicted ones. A number of spectra (dispersion 10 Å/mm) were obtained with the 32-inch camera of the 100-inch coude spectrograph, using both gratings, for spectrophotometric analysis in the region 6800–3700 Å. Normal G-K giants were also photographed as standards. The spectra were calibrated both by means of the wedge strips and by means of the auxiliary wedge-slit spectrograph. The two calibrations, plotted against each other, showed good agreement in all cases; consequently the wedge-slit calibrations have been used, and all the spectra have been traced with the Babcock direct-intensity microphotometer. Wavelengths have been measured and identifications completed in the photographic region of both the peculiar and the standard stars, and measurement of equivalent widths is currently under way. In the visual region, wavelengths have been measured and identifications are now being made.

Reductions and analysis were carried out by the Burbidges on a number of coude spectrograms, obtained at the McDonald Observatory, of five stars having some of the characteristics of Population II. Abundances of the elements Mg, Al, Ca, Sc, Ti, Cr, Mn, Fe, Sr, Y, Zr, and Ba were obtained relative to the standard star 95 Leonis. They were found to range from

nearly normal values to underabundances similar to those found by J. W. Chamberlain and L. H. Aller in two Population II subdwarfs.

Spectra of two high-velocity stars, HD 86986 and HD 103095, taken from Miss Roman's list, and of the standard star 95 Leonis, were obtained with the 100-inch coude spectrograph. Microphotometer tracings have been made, but reductions have not yet begun.

G. R. Burbidge has carried out an analysis of the spectra of the peculiar A star HD 151199, which had been obtained at the McDonald Observatory. Abundances, relative to the standard star 95 Leonis, were normal with the exception of Mn, Sr, and probably Eu, all of which were found to be overabundant.

According to the work of Dr. William A. Fowler and the Burbidges, nuclear reactions may occur in the atmospheres of the peculiar A stars. These reactions will produce positrons, which will then combine with electrons to form positronium. The expected abundance of positronium is too low to produce spectroscopic effects, according to Fowler and the Burbidges. A study by Deutsch of an infrared coude spectrogram of  $\beta$  Coronae Borealis has verified the expectation that the positronium Balmer series does not appear in this spectrum.

A curve-of-growth analysis has been carried out by G. R. Miczaika, F. A. Franklin, Deutsch, and Greenstein for the two metallic-line stars 8 Comae Berenices and 15 Vulpeculae. As Greenstein first found for  $\tau$  Ursae Majoris, the atmospheres of these main-sequence stars simulate those of supergiants, with high turbulence, and low electron pressure and opacity. Abundance anomalies similar to, but generally smaller than, those in  $\tau$  Ursae Majoris occur also in these two stars.

The lines of Tc I have been found by Merrill in the spectrum of the N-type star 19 Piscium. A search for Tc lines in class R stars by Sanford led to negative results.



## STELLAR MAGNETIC FIELDS

The program of observations of stellar magnetic fields has been continued by H. W. Babcock. In an attempt to explore the relations between physical parameters, further groupings of the magnetic A-type stars according to regularity or irregularity and to reversal or nonreversal of polarity have been made. For all such stars that have been adequately observed the effective magnetic field has been found to vary, and all the spectra are to some degree peculiar in that abnormal abundance relations seem to obtain among the chemical elements. In 20 of these stars, the magnetic fluctuations are irregular; about 10 of them show occasional reversal of polarity of the field, and the others do not reverse. Another group of 6 variables shows periodic variations of the field, and only for these are there appreciable variations in the line intensities. But among the magnetic stars, whether regular or not, there is a general tendency for the degree of spectrum peculiarity (abundance anomaly) to increase with the amplitude and frequency of the magnetic variations. This finding supports the concept, suggested some years ago, that surface nuclear reactions, resulting from ionic acceleration in the changing magnetic fields, are responsible for the abundance anomalies.

A survey of line widths indicates that some of the known magnetic stars have the sharpest lines (smallest rotational broadening) of all the numerous A-type stars. Indeed, after a ten-year search for sharp-line stars, 20 have been found with line widths less than  $0.2\text{\AA}$ ; of these, 18 have measurable magnetic fields and only two are "normal."

A study of the large-amplitude magnetic variations of HD 71866, based on 61 spectrograms made with the differential polarizing analyzer, has been completed. The period, which is probably slightly irregular, is within the range 6.798–6.800 days, and the effective magnetic amplitude of the reversing field is about 2 kilogauss. The

star is a spectrum variable of only low degree, and shows random fluctuations of velocity without definite evidence of harmonic variations. The crossover effect on the line profiles of Fe I is more prominent than in any other star observed, but it is far less in evidence for lines of the ionized elements. On the reasonable assumption that visibility of the effect is near maximum owing to a matching of the Zeeman displacements by Doppler displacements for two hypothetical areas of opposite magnetic polarity, the matching velocity derived from several lines of Fe I is about 5 km/sec. On the "rotator model," this would lead to a  $v_e$  (axial rotation) of 3.5 km/sec, whereas an A0 star having a period of axial rotation of 6.8 days should have  $v_e=16$  km/sec. Furthermore, the line widths in HD 71866, by direct measurement, are appreciably narrower than would be indicated by the rotator model.

It is noteworthy that a fairly strong and variable magnetic field with reversal of polarity has been detected in the cluster-type light-variable RR Lyrae by H. W. Babcock. The available observations are insufficient to establish the elements of magnetic variation, but large changes occur from day to day. The occurrence of a coherent magnetic field in a cluster-type variable is significant for the theory of stellar evolution because of the critical position of these variables in the evolutionary scheme of Population II objects.

G. R. Burbidge has investigated the effect of such a field on the pulsation of RR Lyrae. It has been shown that, for field distributions such that  $H_{\text{central}}/H_{\text{surface}}=10^3$ , changes of the period of a few per cent can be expected. The effect of a strong magnetic field on a degenerate stellar core is also under investigation.

There is some reason to believe that the periodic spectrum variables of type A, and the magnetic variables among them, are spectroscopically patchy stars in rigid rotation. On this model, it may be possible to explain the observed changes in line strength, line width, radial velocity, and

Zeeman effect as due simply to aspect changes accompanying the stellar rotation around an axis inclined to the line of sight. In order to explore the adequacy of such a model, the magnetic potential and the abundance anomalies have been developed in spherical harmonics, and the Laplace coefficients of these expansions have been related to the Fourier coefficients of the observed curves, which give line strength, radial velocity, and effective field as a function of phase. With this analytical apparatus, Deutsch has been able to derive a rigidly rotating configuration that is quantitatively in fairly close agreement with the observations of HD 125248. In principle, the configuration that has been derived is uniquely determined by the observations. Existing observations of  $\alpha^2$  Canum Venaticorum are being considered with the same theory. The end result of this harmonic analysis will be a unique map both of the abundance irregularities on these stellar surfaces and of the associated magnetic fields.

#### OTHER STUDIES

In 1954, Wilson showed that there is a relation between the widths of the H and K emission components of Ca II in late-type stars and the absolute magnitudes: the brighter the star, the greater the width. With this encouragement, nearly all the MK standard stars later than G0 in the 1953 list of H. L. Johnson and W. W. Morgan have now been observed at a dispersion of 10 Å/mm. More than 200 spectrograms of about 150 stars have been measured by Wilson, and the results are highly interesting. When the measured widths are corrected for instrumental width, it is found that a plot of  $\log W$  against  $M_V$  defines a straight line. The internal consistency of the measures is such as to suggest that most of the scatter in the diagram is due to erroneous values of  $M_V$ . If this proves to be so, the method should provide a very powerful tool for determining the luminosities of stars bright enough for observing H and K with adequate dis-

persion. The linear relationship extends at least from  $M_V = +9$  to  $M_V = -6$ , and probably even farther. This work is a joint project with Dr. M. K. V. Bappu, who took some of the spectrograms in 1951-1952.

The  $\lambda 5876$  line of He II has been found by Wilson and Dr. M. K. Aly to be present as a diffuse absorption in the spectra of G- and K-type stars which have exceptionally strong central emission components of H and K of Ca II. The search for this line was suggested by analogy with solar plages.

The star HZ 22 is a single-lined spectroscopic binary; its period has been found by Greenstein to be near 4 days, semiamplitude 125 km/sec, and minimum mass near 6 solar masses. This must be a runaway Population I star about 5000 parsecs above the galactic plane.

It has been shown by Deutsch that the M5 II star  $\alpha$  Herculis and its visual companion are enveloped in an expanding gas cloud with a diameter at least 150,000 times that of the sun. This gas has been expelled from the M star and is being lost into the interstellar medium at a rate of at least a solar mass in  $3 \times 10^7$  years. Since the shell-type absorption lines of this envelope are characteristic of all M stars more luminous than normal giants, it is probable that all these objects are losing mass at a comparable rate. By extrapolation of the observed rate of mass loss, and the observed space density of M giants, backward for  $5 \times 10^9$  years, it is found that the number of M giants that have "died" is nearly equal to E. E. Salpeter's estimate of the total number of "dead" stars of Population I. This is the number of stars that have populated the main sequence above  $M_V = +3.5$ . The remnants of these originally massive stars are probably the white dwarfs. Half or more of the present interstellar gas may once have been in M giants. A large fraction of the He, C, N, O, Ne, and the stable elements heavier than Cu, which are now in interstellar gas and early-type stars, may have been synthesized in

the hot cores of these past generations of M giants.

Work on the synthesis of the chemical elements in stars has been continued by G. R. Burbidge in collaboration with Fred Hoyle and with W. A. Fowler, R. F. Christy, and E. M. Burbidge, of the Kellogg Radiation Laboratory. It has been suggested that the spontaneous fission of the transuranic element californium 254, with a half-life of 55 days, is responsible for the form of the decay light-curves of supernovae of type I, studied and observed by Baade, which also have an exponential form with a half-life of 55 days. The way in which  $\text{Cf}^{254}$  may be synthesized in a supernova outburst and reasons why the energy released by its decay may dominate all others have been studied. This work has paved the way for a detailed attack on the synthesis of the elements in stars. It is now believed that with very few exceptions it is possible to account for the abundances of all the elements from H to U. A number of separate astrophysical conditions are demanded, including the increase of temperature in the core of a giant star to about  $10^8$  degrees, which will lead to the synthesis of  $\text{C}^{12}$ ,  $\text{O}^{16}$ ,  $\text{Ne}^{20}$ , etc. Neutron production through the  $\text{Ne}^{21}(\alpha, n)\text{Mg}^{24}$  reaction in the core of giant stars at temperatures of  $2 \times 10^8$  degrees will synthesize elements by  $(n, \gamma)$  capture processes on a slow chain with a time scale  $\sim 10^5$  years. Neutron production by the same reaction at  $2 \times 10^9$  degrees will lead to the synthesis of many isotopes heavier than Fe, including U, by  $(n, \gamma)$  processes on a fast chain with a time scale  $\sim 10$  seconds. This synthesis is believed to go on in supernova outbursts.

The elements between Ti and Cu may be synthesized by an equilibrium process at temperatures between 3.8 and  $5 \times 10^9$  degrees; this synthesis is believed to occur in the presupernova stage. The reactions  $(\alpha, \gamma)$ ,  $(p, \gamma)$ , and possibly  $(\gamma, n)$  on a fast time scale are demanded to synthesize such isotopes as  $\text{Si}^{28}$ ,  $\text{S}^{32}$ ,  $\text{A}^{36}$ ,  $\text{Ca}^{40}$ , and the proton-rich isotopes of the heavy elements. D, Li, Be, and B may be produced by spallation reactions and other processes in the surfaces of stars.

The consequences of the existence in the universe of antimatter, consisting of antiprotons and positrons, have been studied by Fred Hoyle and G. R. Burbidge. When protons and antiprotons collide they annihilate each other with the emission of a number of mesons. These decay, giving rise to neutrinos, antineutrinos, gamma rays, electrons, and positrons. Of these particles, the effects of only electrons and positrons will be detectable. Depending on the density and magnetic field in the region in which they are produced, they will lose energy either by atomic-collision processes which will tend to heat the surrounding medium, or by radio emission. By considering the effect of these electrons and positrons on the interstellar gas in our Galaxy it has been shown that the ratio of antimatter to matter cannot exceed  $10^{-7}$ . The importance to radio astronomy of such a limit's being attained has been investigated. It appears that the intensities of radio emission from galactic and extragalactic sources may be quantitatively explained on this hypothesis. The relation of these ideas to cosmology is also being studied.

## GASEOUS NEBULAE AND INTERSTELLAR MATTER

### INTERNAL MOTIONS

Wilson and Münch have continued their program on the internal motions in the Orion nebula. The observation of fields in the nebula with the 6-foot coude camera and multislit at Palomar is virtually finished, and the measurement of these plates

by Mrs. Coffeen and Miss Flather is about half completed. Intercomparisons among various measurers have shown that personal systematic errors are small. The fitting together of plates of adjacent fields which overlap, however, presents difficulties, apparently because the dispersion of



4.5 A/mm is sufficient to show structure in the nebular lines in many regions apart from those where the lines obviously double. Hence, if the overlapping exposures are not of precisely the same density, the centers of gravity of the lines on the two plates do not exactly agree. No method of correcting for these discrepancies has yet been found.

#### DENSITIES AND CHEMICAL COMPOSITION OF NEBULAE

Osterbrock, in collaboration with M. J. Seaton, of the University of London, investigated the use of the intensity ratio of the  $\lambda 3729$  and  $\lambda 3726$  lines of [O II] for the determination of densities in gaseous nebulae. The agreement between the computed and observed values of this ratio in low-density objects (H II regions) is excellent. In high-density objects (dense planetaries) the observed value of the ratio is somewhat smaller than the computed value, so that a slight correction to the transition probabilities is necessary. A formula based on these corrected transition probabilities and on the calculated collision cross sections may then be used to determine the density in other objects, such as typical planetary nebulae and bright rims in diffuse nebulae. A comparison of the density of NGC 7027 obtained by this method with values obtained by other methods, including surface-brightness measurements, shows that this planetary has a strongly nonhomogeneous structure with pronounced density fluctuations. The  $\lambda 3729/\lambda 3726$  ratios in some of the bright filaments in the Cygnus loop and in the brightest filament in IC 443 show that these objects have densities in excess of  $10^3 \text{ cm}^{-3}$ . Photometrically calibrated direct plates of several planetaries were obtained, to be used, together with already observed  $\lambda 3729/\lambda 3726$  ratios, to determine the density distributions in these objects.

A study of the "elephant trunk" or "comet tail" structures in diffuse nebulae

was begun by Osterbrock. The 48-inch schmidt plates taken for the National Geographic Society-Palomar Observatory Sky Survey were supplemented by a number of additional 48-inch schmidt plates taken during 1955 especially for this program. The dimensions and orientations of a large number of elephant trunks in nebulae of known distances were measured, and a classification of the forms of these trunks was made. These dimensions and forms, as observed, are in good agreement with the theory proposed and developed by H. C. van de Hulst, L. Spitzer, Jr., E. Friedman, and D. Layzer to account for the formation and evolution of these trunks. In all cases the trunks point to the exciting O star of the nebula with fair accuracy, and from the directions of a number of trunks the O star may often be located independently of other evidence. This method appears to be applicable to the galactic-structure problem of locating distant spiral-arm indicators. The density of the bright rim of the elephant trunk in NGC 2264 was found to be  $4 \times 10^2 \text{ cm}^{-3}$  by observations of the  $\lambda 3729/\lambda 3726$  ratio, a density considerably higher than that of the nebula as a whole, and the density in the dark part of the trunk is presumably even higher. Likewise, direct photographs of NGC 6523 and NGC 6611 taken with the 48-inch schmidt show that, although in the light of  $\text{H}\alpha + [\text{N II}]$  the bright rims of the elephant trunks are easily visible, they almost completely disappear in the light of [O III]. This observation is additional proof of the high density in the trunks.

A determination of the helium-to-hydrogen ratio in the central region of the Orion nebula by means of a spectrophotometric study of the hydrogen and triplet-series He I lines was completed by J. Mathis. The theory of the population and equilibrium in the helium levels was developed. The ratio He/H, by number of atoms, was found to be near 0.14.

NEBULAE AND EMISSION STARS IN THE  
SOUTHERN HEMISPHERE

During the year 1955-1956, Henize continued cataloguing southern emission-line stars and nebulae found on objective-prism photographs taken with the Mount Wilson 10-inch camera while it was operated by the University of Michigan at its Lamont-Hussey Observatory in South Africa.

Seventeen short-exposure (limiting magnitude=8.0) and eight medium-exposure (limiting magnitude=11.5) plates were searched during the year. The latter were plates taken in Cygnus and northern Sagittarius to provide a comparison of the southern survey with the northern. This work completed the search of all short- and medium-exposure plates taken in the southern H $\alpha$  survey.

A total of 1493 new emission-line objects (not including diffuse nebulae) have been discovered in the southern survey, including the comparison region in northern Sagittarius. Positions have been measured for all objects, descriptions of emission-line characteristics have been made, and the data have been punched in IBM cards.

A preliminary analysis of these emission-line objects gives the following distribution of types.

Be stars without HD spectral classes...	645
B, A, F, and G stars with HD classes...	605
Stars with special peculiarities.....	27
Probable planetary nebulae.....	115
Possible planetary nebulae.....	54
T Tauri stars.....	8

Probable T Tauri stars.....	21
Wolf-Rayet stars .....	2
Probable Wolf-Rayet stars.....	16
Total .....	1493

Data for the region in Cygnus are not yet complete. Approximately 90 new emission-line objects have been found. Of these, 5 are new planetary nebulae whose character has been confirmed by direct plates taken with the 60-inch reflector.

## INTERSTELLAR ABSORPTION LINES

The observations for Münch's program on interstellar lines have been completed for the stars in the northern Milky Way, and are now being extended to the region south of galactic longitude 160° near the galactic center. In the latter region are found the most distant galactic B stars known, according to W. A. Hiltner and B. Iriarte. A few of them have been observed with the 36-inch camera of the 200-inch coude spectrograph.

Sanford has made a search for a suspected interstellar band near 4770Å on microphotometer tracings of early B stars that are reddened or have strong interstellar bands at 4430Å. For the most part, available spectrograms were not suitable for tracing in this region. Although a mean wavelength of 4769Å was derived for the band from about 10 stars, the evidence is weak, and there is no certainty that it corresponds to a constant velocity.

## GALAXIES

THE ANDROMEDA GALAXY (MESSIER 31) AND  
OTHER MEMBERS OF THE LOCAL GROUP

Baade's investigation of cepheid variables and other distance indicators in the Andromeda galaxy was essentially completed during the report year. For this program Miss Swope finished the determination of the photographic light-curves in the outer Andromeda field 96' south preceding the nucleus; although the spiral arm crossing

the field is poorly populated, 20 of the variables turned out to be cepheids with periods ranging from 21 to 2.5 days. They should furnish the final value for the distance modulus of M 31, because they have been observed both photographically and photovisually, so that, from the color excesses, allowance can be made for any absorption effects. The transfer of the photovisual scale from Selected Area 68 is well



under way and should be finished during the coming season.

At the Eichner photometer Miss Swope has nearly finished the measures of about one hundred cluster-type variables in the center region of the Draco system. The derivation of the periods and light-curves will start within the next two months. During the past season the necessary plates have been obtained by Baade for the construction of the color-magnitude diagram of the Draco system.

Both the Leo and Ursa Minor systems are still under observation by Baade. The Leo system requires excellent seeing conditions because its cluster-type variables lie close to the limit of the 200-inch. Progress in the observation of the variables of the Ursa Minor system had been slow during past years on account of the mirror troubles mentioned under Instrumentation, below. Since the adjustment of the mirror supports, the accumulation of the necessary plates has proceeded at a normal rate.

The long-contemplated study of M 33, which is the nearest spiral member of the local group, was started by Sandage in August 1955. M 33 is, in many respects, a better object than M 31 for the study of stellar contents because of the lack of appreciable obscuration, the larger tilt angle to the line of sight, and the openness of the spiral arms. A series of plates was taken in two colors with the 200-inch for a study of the cepheid variables in an arm south preceding the nucleus. Plates sensitive to the yellow spectral region were also obtained over the entire face of the galaxy. The Population II stars were easily resolved. Inspection of these plates revealed more than a hundred globular clusters, only four of which were known before. Plates sensitive to  $H\alpha$  were also obtained for a study of the size distribution of the  $H II$  regions. These studies of M 33 are planned as the first step in the attempt to obtain a definitive value of the expansion parameter  $H$  of the velocity-distance relation, and hence the distance scale.

## VELOCITIES AND DISTANCES OF GALAXIES

The redshift-magnitude relation for galaxies was reported on last year. One of the major conclusions of this work was the apparently significant departure from linearity of the larger redshifts as obtained from cluster galaxies. If confirmed, this departure would indicate a deceleration of the expansion of the universe. To test this result the current redshift program is confined to observations of faint galaxies in the most distant clusters that can be found and observed. Two such clusters, whose predicted redshifts are greater than 100,000 km/sec, have already been observed by Humason. Measurements of the spectra obtained showed that the shifts for both are probably large and of the right order. The results must be confirmed, however, and the observations will therefore be repeated during the coming year with extended exposure times.

The program for the development of a new distance criterion for galaxies which is based on their apparent angular dimensions and which will therefore be free of errors due to space absorption has been continued by Baum with the photon-counting photometer on the 200-inch telescope. Since each galaxy is a fuzzy object whose outskirts taper off indefinitely, the luminosity measures yield a "profile" which provides both an index of apparent luminosity and an index of apparent size. All together, photoelectric profile observations have now been obtained for about twenty elliptical galaxies belonging to five large clusters whose redshifts fall in the range between 0 and 0.2c (60,000 km/sec).

In addition to profile data, spectral-energy distribution curves  $E(\lambda)$  are also being obtained by Baum for some of the same objects. At present, these curves are based on photon counts in six colors, much of the new material being added by the Farnsworth red-sensitive photomultiplier described in the report for last year.

These spectral-energy curves have recently assumed a new significance. Data

obtained several years ago by J. Stebbins and A. E. Whitford appeared to show that distant elliptical galaxies are redder than would be expected from the redshifts determined spectroscopically. Whitford's six-color results of 1955 indicate that this earlier conclusion was incorrect; after corrections for redshifts are applied, distant galaxies are found to have roughly the same intrinsic colors as galaxies of similar types near by. It follows that the redshift is evidently the primary cause of reddening, and that reddening becomes in fact a measure of the redshift. This is an important development, because photoelectric  $E(\lambda)$  curves can now yield redshifts for remote galaxies beyond the present spectroscopic range. Indeed, there is an unexplored range of 3 or 4 magnitudes between the spectrographic limit and the faintest galaxies measurable photoelectrically, and it is now clear that observations within that range will be vital to any definitive cosmological result.

$E(\lambda)$  observations have thus far been obtained for ten galaxies in five clusters, two of which have redshifts considerably greater than 0.2c. Although large redshifts determined in this way are uncertain by the order of 10 per cent, there is no reason to press for greater precision when the uncertainty in the distances of the clusters involved tends to be at least as great. Spectrographic redshifts, as far as they go, are fully ten times more precise than necessary.

Sandage and Fred Hoyle have applied well known theoretical equations of relativistic cosmology to point out the consequences of the observed departure from linearity in the velocity-distance relation reported last year. If the observed departure is real, a decision can be made between the exploding or evolving cosmologies of conventional relativity and the steady-state universe of Hoyle and of H. Bondi and T. Gold. The present observations are in conflict with a steady-state universe. They require a conventional expanding model of finite dimensions with a positive radius of curvature. The expansion will eventually stop, and contraction will set in. The uni-

verse is of the so-called oscillating type. Our present observations are still too crude to verify these conclusions. When Baum's current program is completed, however, definite evidence may be available.

#### ROTATION OF GALAXIES

Münch has observed the spectra of four of the brighter H II regions near the apparent major axis of M 81 in the Ha region, using the 8-inch camera of the 200-inch coude spectrograph. Rotational symmetry of the system being assumed, the radial velocities of these H II regions give for the radial velocity of the center of mass of the system  $V_0 = -45$  km/sec, in comparison with the value  $V_0 = -55 \pm 30$  km/sec given in the Humason-Mayall-Sandage catalogue. The spectra of fainter emission patches are being obtained with the prime-focus spectrograph. The radial velocity of an emission patch discovered by Baade at a distance of  $17'$  from the nucleus, nearly on the major axis, provides a reliable estimate of the mass of the system. Adopting the distance  $r = 2.6 \times 10^6$  parsecs derived by Sandage, and assuming Keplerian motion, a mass of  $9.7 \times 10^{10}$  solar masses is obtained. In comparison, the motion of the bright H II regions at about  $6'$  (5 Kpc) from the nucleus, if assumed to be Keplerian, gives  $6.8 \times 10^{10}$  solar masses.

The galaxies M 33 and NGC 4594 are also being observed with the Newtonian spectrograph of the 100-inch telescope, for a redetermination of their mass.

#### CLUSTERS OF GALAXIES

Abell has prepared a catalogue of about 2700 rich clusters of galaxies found on the Sky Survey. For each cluster are given the equatorial and galactic co-ordinates, an estimate of the richness, and the approximate magnitude of the tenth-brightest member. From the catalogue is chosen a statistical sample of about 1700 clusters each of which contains at least 50 members brighter than 2 magnitudes beyond that of the third-brightest member. The sample

is being used to investigate the isotropy and uniformity of the distribution in space of the rich clusters of galaxies.

Zwicky has continued his program on the statistics of clusters of galaxies. Contours of all clusters are being plotted on charts which contain all the galaxies brighter than  $m_p = +15.5$  as determined with the 18-inch schmidt. Each chart is accompanied by a list of galaxies with positions and magnitudes as well as by a list of clusters with positions, populations, compactness index, and estimated distance. Work on about 200 clusters has been completed so far.

More evidence has been obtained that the richest spherical clusters at all distances that can be reached at present have approximately the same populations and the same internal structures. There is no evidence for any evolution of clusters. The study of the structural indices has led to a successful method of predicting the red-shift for distant clusters, based on counts of cluster galaxies on plates taken with a series of exposure times. The highest number of galaxies counted in the centers of the most distant clusters photographed with the 200-inch amounts to 300 galaxies per square minute.

Zwicky has continued photographing and analyzing the previously reported extended luminous intergalactic clouds within various clusters of galaxies. Comparisons of the counts of distant galaxies and clusters of galaxies in slightly obscured regions of the Milky Way and in regions covered by near-by clusters have confirmed the conclusion that the loss of light traversing a rich compact cluster of galaxies may amount to half a magnitude.

Tentative photon-count measurements obtained last year indicated that seemingly "vacant" patches between galaxies in a cluster were measurably brighter than similar patches outside the cluster. This result was checked by Baum during the report year by additional observations made

with an improved technique. Within the central regions of clusters, the surface brightness of "vacant" patches (relative to those outside the clusters) is found to be around 27th photovisual magnitude per square second of arc. Although this amounts observationally to only 1 per cent local increment in total sky brightness (including the airglow, etc.), it adds appreciably to the total light associated with the cluster itself, and it provides a crude quantitative indication of the extent to which the outskirts of galaxies fill in the spaces between them.

#### INTERCONNECTED MULTIPLE GALAXIES

Attempts were made by Zwicky to discover any possible polarization of the light from the most conspicuous intergalactic filaments connecting the members of certain multiple galaxies. No polarization has been found so far. This negative result, in conjunction with the fact that no emission lines have been observed in the spectra of the filaments, strengthens the conclusion that these filaments are composed of stars. More filaments were photographed with the 200-inch. It was also found that most close double galaxies which from independent criteria appear to be physical doubles also show extended horns representing tides and countertides.

#### STUDIES OF INDIVIDUAL GALAXIES

The photographic photometry of all galaxies brighter than  $m_p = 15.0$  was continued by Zwicky. Likewise more blue and red plates were taken with the 48-inch schmidt to cover the region from R.A.  $12^h$  to  $14^h 40^m$  and Decl.  $+5^\circ$  to  $+15^\circ$  for a study of the distribution of galaxies carried out in co-operation with the Lick Observatory.

The method of composite photography was extended to include polarization, and positive results are indicated in NGC 5128, 5194, 3034, and 4565.



## RADIO SOURCES

POLARIZATION IN THE CRAB NEBULA AND THE  
JET OF MESSIER 87

One of the outstanding events of last year was the explanation of the light-emission in the Crab Nebula as due to electrons circling in a magnetic field (synchrotron mechanism). Since, according to this theory, polarization effects up to 100 per cent were to be expected in the continuum of the Crab Nebula, a test at the 200-inch telescope seemed especially desirable on account of the detailed information provided by its large-scale pictures. Working in the wavelength range  $\lambda 5200$  to  $\lambda 6400$ , in order to suppress the strong emission of the outer filaments of the Crab Nebula, Baade obtained in September 1955 a set of polarization pictures of the continuum which proceeds in steps of  $22.5^\circ$  of the polarizing angle. The striking polarization effects revealed by these pictures approached in several places the predicted 100 per cent. It is also obvious that the details visible in the central mass (arcs and soft wide bands) reflect the run of magnetic lines of force and that the whole central mass is a superposition (in depth and angles) of a complicated system of lines of force. Since with proper orientation of the analyzer each of these structures can be made to stand out with maximum contrast among the confusing superposed features, it will be possible in the future to study the motions of the central mass almost as easily as those of the outer filaments.

Baum also made measurements of the polarization of small patches in the Crab Nebula with a polarizing attachment fitted to the photoelectric photon counter on the 200-inch Hale telescope. All together, five small selected patches were measured, the smallest being  $7''$  of arc across, and the most strongly polarized showing about 70 per cent polarization in the yellow part of the spectrum.

Similar studies of the polarization of the Crab Nebula were made by Zwicky, using

his method of composite photographs. In this procedure a positive print of a photograph of the nebula taken through a Polaroid filter is superimposed on a negative print taken through a Polaroid oriented perpendicular to its position when the photograph for the positive print was exposed.

Since there were good reasons to suspect that the well known jet in M 87 is another object in which the synchrotron mechanism is operative, its polarization was tested by Baade at the 200-inch. The procedure was the same as that for the Crab Nebula. Nothing can be said as yet about the polarization of the thin jet itself, because in the wavelength range used it is drowned in the strong light of the center region of M 87. The three strong condensations in the outer part of the jet are clearly polarized, however. The effect is most striking in the two innermost condensations, which form a close pair, because they exhibit a phase shift of about  $90^\circ$  in their polarizations. To obtain quantitative measures it will be necessary to repeat the tests under optimum seeing conditions with a greater focal length.

## NEBULOSITY NEAR POSITION OF NOVA OF 1572

Experiments have been started with the 48-inch schmidt telescope on the use of a red filter which is more restricted to  $H\alpha$  than the red Plexiglas used for routine exposures. In the course of this work, a very faint nebosity has been discovered by Minkowski near the position of Tycho's nova of 1572, and of a known radio source. This nebosity is under more detailed investigation. It is undoubtedly the remnant of the nova, which until now was the only one of the three known galactic supernovae whose remnant had not been found.

## INTERNAL MOTIONS IN THE CRAB NEBULA

To find a possible connection between the magnetic field in the amorphous mass of the Crab Nebula, as revealed by the polarization measures, and the motion of

the filaments, a series of spectra (110 Å/mm) was secured by Münch with the Newtonian spectrograph of the 100-inch telescope. The measurement of these plates, however, has given results of accuracy lower than was expected, owing to an unsatisfactory comparison spectrum, and the whole series will be repeated in the coming season. In order to verify that the lines observed near the position of the Balmer lines are not contaminated appreciably by those of deuterium, which might be produced in the high-energy processes, spectra of one of the brighter filaments were obtained with the 8-inch camera of the Palomar coude spectrograph. From the measures of two plates it has been found that the velocity of the H $\alpha$  lines agrees with that of the neighboring [N II] pair within a probable error of only 6 km/sec. A stronger exposure, however, is needed to establish an upper limit for D/H of the order of 1/100.

#### MECHANISMS OF EXCITATION OF LIGHT AND RADIO EMISSION

Greenstein has considered the problem of the source of the relativistic electrons which, when accelerated by synchrotron action in the magnetic field of the Crab Nebula, are the origin of the polarized light and radio noise of this object. Two injection mechanisms have been proposed: one, the decay of mesons produced by spallation of heavy cosmic-ray particles; another source may be electrons from deuteron-deuteron or deuteron-proton reactions in colliding gas masses. This process produces electrons of a few million electron volts, and may be important in the Crab Nebula.

A theoretical study of the relation between thermal radio noise and the surface brightness of diffuse emission nebulae has been carried out. With proper allowance for optical interstellar absorption, the radio noise at high frequencies seems to be largely thermal in origin.

The energy and magnetic-field relations necessary to explain the observed polarized

light and radio emission in the jet in M 87 have been investigated by G. R. Burbidge. To account for the optical radiation in the jet, the total energy required in particles and field ranges from about  $10^{54}$  to  $10^{59}$  ergs for a series of assumed magnetic fields ranging from  $10^{-2}$  to  $10^{-6}$  gauss. To account for the radio emission for the same series of magnetic-field strengths, total energies in the range  $10^{56}$  to  $10^{61}$  ergs are demanded. It is suggested that reasonable minimum values may be about  $2 \times 10^{55}$  ergs in particles and field, with  $H \sim 10^{-3}$  gauss in the jet, and about  $10^{57}$  ergs in a volume 200 times greater than that of the jet, also with  $H \sim 10^{-3}$  gauss, and that this will explain the radio emission. By considering nuclear collisions of the high-energy protons with the static material in the jet, it is shown that a continuous supply of electrons and positrons will be produced. If a density of about  $3 \times 10^{-22}$  gm/cc is assumed in the jet, the rate of electron-positron energy generation will be sufficient to balance the loss of energy by synchrotron radiation. The jet will also be a source of high-energy gamma radiation.

A theoretical study of the sources of radio emission in NGC 5128 and NGC 1316 has been made by E. M. Burbidge and G. R. Burbidge. These are fairly similar radio sources which have two distributions of radio emission, a central region with roughly the dimensions of the optical galaxy and a halo of much greater extent. From information supplied by J. G. Bolton, of the California Institute of Technology, and on the assumption that the emission is synchrotron radiation, the possible ranges of total particle and magnetic-field energies have been calculated. If electrons and positrons form the major part of the total particle flux, the minimum values of total energy and effective magnetic field are about  $10^{55}$  ergs and  $10^{-5}$  gauss in the central regions and  $10^{57}$  ergs and  $10^{-6}$  gauss in the halo regions. If the electron-positron flux is derived from a proton flux of larger total energy, the minimum values are about  $10^{57}$  ergs,  $2 \times 10^{-4}$  gauss, and  $10^{59}$  ergs,  $10^{-5}$



gauss, in the central and halo regions, respectively. Possible theories of origin of the particle flux, including the annihilation of antiprotons by protons, and interpretations of the nature of the galaxies have been considered.

The radio emission in our own Galaxy and its connection with the cosmic-ray flux have been studied by G. R. Burbidge. The halo distribution of radio emission observed both in our Galaxy and in M 31 is explicable if a very low density of gas ( $\rho=10^{-26}$  to  $10^{-27}$  gm/cc) is present together with a magnetic field of about

$2 \times 10^{-6}$  gauss. This gas must be in highly turbulent motion ( $v \sim 200$  km/sec). The radio emission has its origin in the high-energy electrons and positrons which are continuously being accelerated by Fermi-type mechanisms in the halo, and which may have originated as decay products of mesons produced in collisions between cosmic-ray protons in the disk of the Galaxy. Calculation of these collision rates suggests that the present flux of cosmic rays is sufficient to produce radio emission of about  $10^{37}$  ergs/sec, which is near the observed value.

## INSTRUMENTATION

### READJUSTMENT OF 200-INCH MIRROR SUPPORTS

In taking direct photographs with the Hale telescope, an  $f/3.67$  Ross correcting lens is generally used in combination with the 200-inch mirror. The Ross lens was designed to give a properly corrected field  $15'$  of arc in diameter. A close watch of its performance during the past three years showed, however, that the actual size of the usable field approached the computed value only on rare occasions. To make things worse, the usable field was on the average not only very much smaller than expected but also decidedly asymmetric with respect to the optical axis. These troubles were particularly pronounced in fields of high declination ( $\delta > +50^\circ$ ), where they were coupled with substantial astigmatism of the mirror which changed rapidly with the hour angle. Obviously the complicated support system of the mirror did not function properly.

In the spring of 1956 a careful study was made of the forces acting upon the mirror in various positions of the telescope. It was found that the mirror was being over-defined for rotation about its optic axis. Furthermore, because of flexure of the defining system, large forces were being exerted on the mirror in certain orientations of the telescope. Because accurate definition of the mirror for rotation about this

axis is not necessary, the defining plates were backed off so that no forces were exerted in any orientation. Since this change and a careful readjustment of other parts of the support system, the usable field of the 200-inch in combination with the  $f/3.67$  correcting lens is identical with the computed one ( $15'$  of arc in diameter), it is symmetrical around the optic axis, and the astigmatism of the mirror in high declinations has almost disappeared. In fact, for zenith distances smaller than  $60^\circ$ , where most direct photographs are taken, it never exceeds 0.05 mm.

### GUIDER AT 200-INCH COUDÉ FOCUS

An improved photoelectric guider has been developed by H. W. Babcock, Rule, and J. S. Fassero for use at the coudé focus of the 200-inch telescope. It can relieve the astronomer of the time-consuming task of manual guiding on long exposure. The function of the rotating knife-edge as a detector of error in centering of the star image is fulfilled by a steel ball rolling at 60 revolutions per second, under the influence of a revolving magnetic field, within a stainless-steel ball race having a diameter twice that of the ball. Deviations of the optical image from the center of revolution of the ball result in the development of an a.c. error signal by a multiplier phototube. The information availa-

ble in the phase and amplitude of error signal is used, after amplification in a single channel, to control the tilt, in two co-ordinates, of a gimbal-mounted quartz plate that functions as a feed-back device in the incoming optical beam. Thus the image is maintained precisely in position despite atmospheric deviations or minor errors in the telescope drive.

#### PHOTOMETRIC EQUIPMENT

Two completely new photometers designed by Baum are being fabricated for the 60-inch and 100-inch telescopes at Mount Wilson, several important modifications and additions have been made to the photon counter on the 200-inch telescope at Palomar, and a new photometer has been finished and put into operation on the 20-inch telescope. In order to avoid early obsolescence, all these new instruments have been designed to facilitate a broad range of future additions and modifications.

Initial experiments were carried out at Palomar by Baum on a new photosensitive surface, known as the multialkali photocathode, which was developed by R.C.A. Laboratories at Princeton; the Laboratories generously made available two of their first handmade multialkali photomultipliers. This photosensitive surface not only is more sensitive than the best photosurface heretofore available, but also spans a much broader range of wavelengths, namely from 3500Å to 8000Å.

These tubes were mounted in a dry-ice chamber to reduce thermionic emission and were tested in conjunction with the photon-counting photometer at Palomar. The multialkali cathodes proved to be about 2.5 times more sensitive at 5000Å and about 50 times more sensitive at 7000Å than the two photocathodes in recent use

at Palomar for these respective regions. Although the experimental multialkali tubes left much to be desired with regard to attainable noise reduction and over-all current amplification, these difficulties are not considered to be fundamental. Some further work will be necessary, however, before multialkali tubes can be put into regular use for routine observations.

#### RULING ENGINE

H. W. Babcock and Swanson have ruled fifteen gratings during the report year. The aim has been to reduce consistently to a still lower level the already minute errors associated with the spacing mechanism, and to develop further the technique of ruling gratings that are highly uniform and properly blazed even though large in area. Comparative tests of Graphitar and Teflon as sliding bearings under the grating carriage have been carried out. A reduction of some 60 per cent in sliding friction does much to recommend Teflon. The reduced friction and consequent reduction in thrust required of the main spacing screw permits the use of a modified end-thrust bearing for the screw that gives some promise of lowering the amplitude of the average periodic error.

A rather unusual large grating for infrared spectroscopy was ruled for Professor John Strong, of Johns Hopkins University, on the older and larger ruling machine. This ruling, 12 by 14 inches in size, was made with a diamond on a specially prepared silver surface with a groove spacing of 40 per millimeter. The blaze was placed in the 39th order of the green mercury line. To achieve the best possible groove form with this coarse spacing, the grating was ruled not once but twice (double-passed) before removal from the machine.

#### GUEST INVESTIGATORS

In continuation of the Observatories' usual policy, a number of guest investigators have been invited from other institutions to make use of the observational fa-

cilities at Mount Wilson and Palomar Mountain. These investigators have carried through the following programs.

The B-star program initiated in 1952 by

Dr. L. H. Aller, of the University of Michigan, with the coudé spectrograph of the 100-inch has been extended by him to include the visual region as well as the photographic regions of  $\lambda$  Orionis, 10 Lacertae,  $\tau$  Scorpii,  $\phi$  Orionis,  $\gamma$  Pegasi, 114 Tauri, 22 and 42 Orionis, 15 Canis Majoris, HD 36959, HD 369690,  $\alpha$  Sculptoris, and a few other stars. Wavelengths, identifications, equivalent width, and line profiles have been investigated for the visual region of  $\gamma$  Pegasi, and reductions are well along for several other stars.

Carefully guided spectrograms of the planetary nebulae NGC 7662 and NGC 7009 were also taken by Dr. Aller with the new 60-inch Cassegrain spectrograph. These failed to confirm Swings' and Swensson's detection of N III emission in the spectrum of the central stars. Observation of AX Persei and CI Cygni with the same equipment shows these stars to be in a high-excitation phase, whereas BF Cygni is in a low-excitation phase with a strong continuous spectrum.

Dr. Dinsmore Alter, of the Griffith Observatory, has continued his study of various lunar features with the aid of photographs taken at the Cassegrain focus of the 60-inch telescope. In order that photographs may be taken without delay if unusually fine seeing occurs, a special plate-holder with a negative enlarging lens has been constructed which may be mounted without removing the spectrograph.

Dr. M. K. Aly, of Helwan Observatory, Egypt, collaborated with Wilson in a search for  $\lambda 5876$  of He I in absorption in the spectra of late-type stars. Since  $\lambda 5876$  is often found to accompany strong H and K emission in plage areas on the sun, spectra were taken of stars showing H and K in emission. The He I line was found in a substantial fraction of the stars observed.

Dr. Aly obtained a few spectra during the ingress of the eclipse of  $\zeta$  Aurigae. These, together with a few spectra taken during egress by Wilson, will be used by Dr. Aly for a study of the 1955-1956 eclipse.

Dr. Arthur Code, of the Washburn Observatory of the University of Wisconsin, continued his program on monochromatic magnitudes of various objects, utilizing a new photoelectric scanning spectrograph of the Ebert-Fastie type. The mechanical parts were built at the University of Wisconsin with the aid of a grant from the National Science Foundation. The spectrograph performed more efficiently than was anticipated, and made it possible to extend the scope of the scanner program.

In addition to bright stars, several other objects of special interest were observed. Photoelectric scans were made of the nuclear regions of M 32 and M 31. In the normal photographic regions both these objects exhibit an energy distribution similar to that of a G8 III star. The luminosity-sensitive lines suggest the normal giant classification for the nucleus of M 31, but probably a Population II type giant for M 32. This new curve for M 32 provides the energy distribution for a standard elliptical for comparison with those of distant ellipticals. It appears that the Stebbins-Whitford effect is essentially removed by the use of this standard. This result is in agreement with the multicolor photometry of distant ellipticals made by A. E. Whitford with the 200-inch telescope in the spring of 1955.

Scanner observations were obtained for emission lines in the planetary nebulae NGC 7027 and 7662, the Orion nebula, and the Cygnus loop. The measurements in the Cygnus loop were in agreement with those found by J. W. Chamberlain and by Minkowski and not compatible with the results of S. B. Pikelner.

The He I 4026 line in 56 Arietis was studied for the rapid variations suspected by Deutsch, and some evidence for changes in equivalent widths over periods as short as a minute was detected.

A study of the Ca II emission lines in the classic cepheid variables, begun at the Lick Observatory, was completed by Dr. Robert P. Kraft, National Science Foundation Fellow. The principal results were the fol-



lowing: (1) The transitory presence of bright H and K is almost universal in cepheids with periods greater than 5 days. (2) With increasing period the bright lines are present in the spectrum a longer time, and the K-line intensity increases. (3) The presence of emission is independent of Eggen type A, B, or C. (4) The time scale for the bright-line phenomenon in long-period variables of type M can be extrapolated from that of the classic cepheids, suggesting that a common activity is responsible for the Ca II emission in the two kinds of objects; the bright lines of the infrared Ca II triplet in the M8e-type long-period variable R Leonis are shown to arise below the level of formation of the TiO absorption bands. (5) A colliding-layer model, advanced to explain the emission lines in classic cepheids, is rejected in favor of a picture consisting of hot gases convected upward low in the atmosphere.

Dr. Kraft, from a 100-inch coude spectrogram taken by O. Struve in 1954, found doubled low-excitation lines of Fe I, Ca I, Sc II, and Ti II in the classic cepheid variable X Cygni (period 16.4 days) at phase 0.82 p. Subsequent 100-inch spectrograms taken by Dr. Kraft confirmed the doubling of these lines and also of H $\alpha$ , the latter showing duplicity from phase 0.76 p at least to 0.87 p. Doubled H $\alpha$  was also found in the classic cepheid TT Aquilae (period 13.8 days) at phases 0.76, 0.78, 0.84, 0.85 p.

A search for new variables of the U Geminorum type was carried out with the Newtonian spectrograph of the 100-inch. T Leonis and EX Hydrae were found to be members of the group. T Leonis is of special interest because of its high galactic latitude. Only one (AY Lyrae) of the fifty members of this group is known to have a pure absorption spectrum at minimum; it was therefore assumed that wide emission lines of H, He, and Ca II are a necessary criterion for membership. The following stars were rejected on this basis:

TU Leonis, SU Canum Venaticorum, and SVS 981 Cygni.

An attempt to detect polarization in the light of SS Cygni at maximum was made by Dr. Kraft with a photoelectric photometer attached to the 100-inch, and a Polaroid plate which could be turned through 30° intervals. Comparison against a nearby field star yielded negative results, with no polarization in excess of 0.015 mg. in the yellow.

Dr. R. B. Leighton, of the California Institute of Technology, investigated solar granulation with the 60-foot solar tower. A 35-mm camera was mounted so as to utilize the 6½-inch solar image formed by the 12-inch lens working at full aperture. On several mornings 10-minute sequences of exposures spaced at about 1-second intervals were taken, using V-G emulsion with an orange filter, just as for the solar patrol plates. In these sequences there appeared several frames on which the resolution was considerably superior to that of even the best plates taken with the 4-inch aperture, and on at least two occasions the resolution was probably equal to the theoretical limit of the optical system.

These photographs indicate clearly that the solar granulation is not a random distribution of bright and dark areas, as has sometimes been claimed, but has a definite cellular structure of bright areas surrounded by relatively narrow boundary lines. A quantitative measure of these features is in progress.

The program of the McMath-Hulbert Observatory for the observation of solar spectra with the Snow telescope has been continued for another year. The observer for the project was Walter E. Mitchell, Jr., up to December 1, 1955. On September 1, 1955, Thomas K. Jones joined the project, and after a period of training made all the observations after December 1.

The program for the current year has been the following: (1) Continuation of the systematic observation of the infrared helium line (10830Å) at the limbs of the sun and in plage regions. (2) Systematic



observation of the central structure of the K line (3934Å) at the limbs of the sun and in the plage regions. (3) Completion by Mitchell of a long series of observations of a number of lines that show conspicuous changes at different points from the center to the limb of the sun. (4) Refined mapping of difficult parts of the solar infrared and visual regions of the spectrum. These regions are needed to complete the solar spectrum map, but can be observed only under unusually good observing conditions. (5) Mapping of infrared sunspot spectra as suitable spots have developed on the solar disk.

Observations were made on 217 days, and a total of 900 tracings was accumulated during the year. The Snow telescope spectrometer was completely inspected, cleaned, and adjusted during Dr. Orren C. Mohler's residence on Mount Wilson in August. A mounting for a Lallemand PbTe cell is under construction and will soon be placed in service to replace a Cashman PbTe cell that was broken.

A fairly long homogeneous series of observations of the central structure of the K line has been accumulated, chiefly at the Snow telescope on Mount Wilson. These structures are closely correlated with intensity changes in the 10830Å lines of helium. The program is at present being changed to include laboratory wavelength standards on the tracings, so that significant wavelength measurements may be made for correlation with the intensity changes of the various components of the K line.

In late August 1955 the publication by Dr. Mohler, *A Table of Solar Spectrum Wave Lengths 11984Å to 25578Å*, was issued. The numerical data in this volume are based wholly on observations made with the Snow telescope from 1949 to 1952. A similar extensive list of identifications for the wavelength region 29000Å to 35000Å is being prepared; it will also make use of data obtained with the Snow telescope infrared spectrometer.

During May and June Dr. D. H. McNamara, of Brigham Young University,

obtained spectrograms of the eclipsing binary stars RZ Scuti and TX Ursae Majoris that will be used for a study of the line profiles, line intensities, and velocity curves of these stars.

Dr. L. Plaut, of the Kapteyn Astronomical Laboratory, Groningen, the Netherlands, has started an investigation of the large-scale structures of the halo of the galactic system. For this purpose he is making a search for variable stars in a few selected regions that lie in the plane, perpendicular to that of the Milky Way, which passes through the sun and the center of the galactic system. Four regions were chosen on the basis of an inspection of Sky Survey plates and of a few plates taken for the purpose by Baade. By June 30, 1956, plates of these regions had been obtained with the 48-inch schmidt telescope, as tabulated.

REGION		NUMBER OF PLATES	
l	b	pg	pV
327.5	+28°	54	13
327.5	-12°	41	13
331.0	+12°	58	14
147.5	+15°	0	0

Dr. Daniel M. Popper, of the University of California at Los Angeles, has continued his spectroscopic studies of eclipsing binaries. Several systems for which there is evidence that earlier measurements need revision have been re-examined, among them S Antliae, RS Canum Venaticorum, RX Herculis, and AW Herculis. Attention has also been given to systems with components above the main sequence. New objects with double lines discovered in the visual region, which give promise of furnishing additional masses above the main sequence, are TV Ceti (period 9 days), RZ Eridani (period 39 days), and VV Monocerotis (period 6 days).

Dr. Otto Struve and Dr. Jorge Sahade, of the University of California, have made extensive studies of a few important spectroscopic binaries and two groups of pulsat-

ing stars: those of the  $\beta$  Canis Majoris type and those designated as dwarf cepheids. Approximately 100 coude spectrograms of  $\beta$  Lyrae were obtained on Eastman Process plates during the summer of 1955. All spectrograms have been measured by Dr. Sahade for the determination of radial velocities. These data should permit a more accurate determination of the orbit than has previously been possible.

Very interesting results have also been obtained on high-dispersion coude spectrograms of the 4-day eclipsing variable  $\alpha$  Virginis. The broad lines of the primary components show interesting changes in profile, especially near the two conjunctions. There is also some indication of structure in these spectral lines at other phases, including the two elongations.

Drs. Struve and Sahade are now concentrating upon the study of the 14-day binary W Serpentis, a star which is surrounded by a shell exhibiting exceedingly sharp absorption lines and many emission lines, including forbidden lines of iron. All spectrograms obtained to date have been measured, and the resulting velocity curve departs markedly from the one previously obtained by C. Bauer at the McDonald Observatory. Among the pulsating stars, 15 Canis Majoris, of spectral type B2, and  $\rho$  Puppis, of spectral type F, have been observed with both the coude spectrograph of the 100-inch and the spectrograph of the 60-inch telescope. The velocity curves of the two stars have been determined. The pulsating star DQ Cephei, also of spectral type F, was investigated in co-operation with O. C. Wilson.

Dr. Struve has also obtained a considerable number of coude spectrograms of  $\epsilon$  Aurigae during its present eclipse. The outcome of this work is a new model of the binary system of  $\epsilon$  Aurigae that removes many of the difficulties of the early Yerkes model. All spectrograms of  $\epsilon$  Aurigae obtained with the coude spectrograph, or by various observers in the interval between the 1928 eclipse and the present one, have been measured at Berkeley. A study

of the velocity curve during this interval is now in progress.

Dr. Uco van Wijk, of Princeton University, observed the red spectra of the supergiants  $\gamma$  Cygni,  $\alpha$  Aquarii,  $\beta$  Aquarii, and  $\alpha$  Persei at the coude focus of the 100-inch in order to measure the turbulence in the atmospheres of these stars from the line profiles. A dispersion of 6.7 Å/mm was used. The two supergiants of slightly later spectral type,  $\alpha$  and  $\beta$  Aquarii, showed considerably less turbulence than the earlier supergiants,  $\gamma$  Cygni and  $\alpha$  Persei.

Dr. van Wijk also obtained red spectra of  $\beta$  Persei, including three spectra taken during primary minimum. These plates were measured by Mr. Meltzer, who found that they showed a faint spectrum due to the third component, an F main-sequence star.

Dr. Sebastian von Hoerner, of the Max Planck Institut für Physik, Göttingen, has investigated the internal structure of globular clusters and has made a frequency study of elliptical, E, and S0 galaxies with the following results:

1. In a typical globular cluster, about half of the mass lies inside the region where the local exchange of energy is sufficient for equilibrium. For these inner parts, a method is given for obtaining the mass-luminosity relation and some information about the distribution of star masses. For six models of central mass distribution, the space-density curves have been calculated. Under the assumption that all stars of the outer parts have been evaporated from the center, the density of the outer parts also could be determined for different star masses.

2. The tidal force of the galaxy is the only force that affects the stability of globular clusters. Less massive clusters should have their limiting radius just within the limit of observation.

3. For M 3 and M 92, space densities of stars of different magnitudes have been calculated from the counts of Sandage and Taylor. The mass-luminosity relation is

close to the form demanded by the theory of stellar evolution. To describe the observed densities, about a thousand stars more massive (1.3–1.5 solar masses) than the observed ones would be necessary (white dwarfs or double stars). In the outer parts there seems to be some original density left over.

4. Baade and Spitzer have shown that in very dense clusters of galaxies the frequency of elliptical, E, and S0 galaxies is very high. This finding was explained by collisions that sweep out the gas. The next question would be whether in the ordinary field also S0 galaxies are made by collisions. If so, there should be a correlation between the frequency of these galaxies and the density of the field. Preliminary classifications and counts on some schmidt plates showed a slight correlation. The present space density is too low to give enough collisions for the observed frequency, but in an expanding universe, the density at an early time (about one-tenth of

the present age) would have been high enough.

Dr. Merle F. Walker, of the University of California, investigated the color-magnitude diagrams of clusters which contain O and B stars and therefore must be extremely young. Three-color photometric observations were made of the clusters H Persei, IC 1805, NGC 2244, NGC 6530, NGC 6611, NGC 6823, NGC 6910, IC 4996, and IC 5146. Reduction of the observations of NGC 6530 has been completed; the color-magnitude diagram resembles that of NGC 2264.

Dr. Walker observed Nova DQ Herculis (1934) for one night under poor conditions. Observations of 15 Canis Majoris with a photoelectric photometer on the Palomar 20-inch reflector confirmed the variability suspected from observations at Lowell and fixed the period at 0.1565 day. This star is thus a member of the  $\beta$  Canis Majoris class having a total variation of 0.02 mag. in yellow light.

## STAFF AND ORGANIZATION

Dr. Walter S. Adams, Assistant Director of the Mount Wilson Observatory from 1913 to 1923 and Director from 1923 through 1945, died at his home in Pasadena on May 11, 1956. Dr. Adams was the last remaining member of the group of young astronomers who came to the Mount Wilson Observatory when it was founded in 1904. Dr. Adams also played a major role in the design and construction of the Palomar Observatory, serving as a member of the Observatory Council from 1934 to 1948, as chairman of the Advisory Committee from 1928 to 1937, and as a member of the Policy and Construction Committees from 1937 to 1948. Finally, he was a member of the Observatory Committee from the start of joint operation of the two Observatories in 1948 until 1950. The Mount Wilson and Palomar Observatories owe much of their present efficiency of plant and effectiveness of organization to Dr. Adams' sound advice based on his broad experience in the operation of mountain observatories.

Miss Alice Beach died suddenly on February 10, 1956. She came to the Observatory in 1929, and was secretary to the Director until 1946, when she became research assistant in the nebular department.

On a trip sponsored by the Radio Astronomy Panel of the National Science Foundation, Minkowski visited most of the existing radio-astronomy installations in the world for discussion and investigation of the problems of identification of radio sources. He stayed for more than two months in Sydney with the Division of Radiophysics of the Commonwealth Scientific and Industrial Research Organization, and made shorter visits at Leiden; Cambridge, England; Jodrell Bank Experimental Station; and Cambridge, Massachusetts.

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## JOINT COMMITTEE ON IMAGE TUBES FOR TELESCOPES

CO-OPERATIVE PROJECT OF MOUNT WILSON AND PALOMAR OBSERVATORIES, DEPARTMENT  
OF TERRESTRIAL MAGNETISM, NATIONAL BUREAU OF STANDARDS, UNITED STATES  
NAVAL OBSERVATORY, AND CALIFORNIA INSTITUTE OF TECHNOLOGY

M. A. TUVE, *Chairman*

*Carnegie Institution of Washington, Department of Terrestrial Magnetism*

In February 1954 the Carnegie Corporation of New York awarded a grant of \$50,000 to the Carnegie Institution of Washington for an exploratory program for the development of image converters to increase the range of large telescopes. A committee set up by Dr. Vannevar Bush for this purpose, under the chairmanship of Dr. M. A. Tuve, also includes the following members: Dr. W. A. Baum, Mount Wilson and Palomar Observatories; Dr. L. L. Marton, National Bureau of Standards; Dr. John S. Hall, U. S. Naval Observatory.

This Committee during the year just past has been able to stimulate and foster several highly technical items of industrial development that may be useful for extending the range of telescopes to fainter objects. Two quite different electronic devices are well along toward sky tests, but real fruitfulness for astronomy is still a matter of hopes for the future.

Developments during the past few years in electronic techniques, especially those used in television, in photoelectric surfaces, and in phosphors which emit light when bombarded by electrons, all have given hope to physicists and astronomers that a marked extension of the usefulness of large telescopes may be possible. Astronomical measurements of objects at great distances involve exceedingly low levels of illumination on the photographic plate or photoelectric surface, even when it is placed in the focal plane of the 200-inch telescope. Hence, applications for astronomy will be very unlikely to come about as an automatic result of industrial television developments, because most industrial uses involve light levels ten thousand

to ten million times greater. Dr. Bush and the members of our Committee recognized in 1953 that a special effort was necessary for astronomy, with technical emphasis on increased contrast and the reduction of random fluctuation, for spot sizes of about  $\frac{1}{10}$  mm, at very low light levels.

The present work has two principal aims, one being to extend the effective distance in space which is accessible to large telescopes (60-inch to 200-inch), and the other to extend spectrum measurements down to much fainter objects. The first aim is basically a matter of increasing the reliability of identification of faint objects that show up against the general background of light from the night sky, much of which comes from the earth's atmosphere. With the 200-inch telescope an object a bit fainter than a 23-magnitude star can be photographed; this means that a local increase of about 15 per cent above the level of the night-sky brightness is detected for a small area ( $\frac{1}{10}$  mm in diameter) of the photographic plate. The hope of the present Committee is that this identification can become definite for local contrasts in illumination of perhaps 2 per cent or less, thus increasing the "distance" to which average objects can be seen, with a correspondingly vast increase in the volume of space that can then be studied, especially for the statistics of color and distribution of distant galaxies. Similar great advantages will accrue if spectroscopic studies can be pushed down from the 12th to the 16th magnitude, for example.

During the past year emphasis has been on the thin-film tubes mentioned in the report of June 1955, and on a new type of internal electron multiplication, suitable

for one-to-one reproduction of the pattern of the light striking an area about 2 cm in diameter, but with an intensity multiplication of a thousand or more, and with a minimum of "graininess" or internal noise added to the original image distribution. A new and apparently practical way to make an image multiplier tube of this kind has been proposed and developed by Dr. E. I. Sternglass and Mr. M. M. Wachtel, of the Westinghouse Research Laboratory, with whom our Committee has been collaborating in discussions and in testing. These two very different types of instrument have both shown great promise in early tests, although technical problems in manufacture have defeated most of the efforts for even pilot-line production to date. No appreciable effort was spent by the Committee during this year to press the development of strictly television-tube techniques (electronic storage image tubes with electrical read-off), although our friend and colleague Professor J. D. McGee at the Imperial College of Science and Technology in London still regards this as a procedure which ultimately should give a great gain (in "signal to noise ratio") and be useful in astronomy.

It thus still appears reasonable to say that in three separate categories or modes of approach to the astronomical problem our early expectations for the possibility of great advantages for astronomy (namely at very low levels of light intensity) have been essentially verified, but the technical problems in fabricating the necessary complex and delicate electronic tubes have defeated nearly all our practical efforts to date. In one of our tests on the 26-inch refractor of the Naval Observatory the image of a selected star was obtained, but most of the photographic plate was fogged by the vacuum gauge and the thin film broke at the start of the next test.

As this report is written (July 1956), two dozen new thin films are in various stages of processing, and it is reasonable to hope that one or several additional thin-film tubes may be tested before the end

of the year. In addition, it is expected that recent fabrication difficulties on the Westinghouse tube may be overcome in time to allow testing of several of their image multipliers in the near future.

Although efforts during many nights were made by Dr. Hall and Dr. Baum to test the few early models of image tubes on the sky itself, using the 26-inch Naval Observatory refractor, a more practical device has been designed and fabricated under Dr. Hall's direction for tests in the laboratory at very low light levels. A photographic negative showing actual stars, with a suitable range of intensities and closeness of spacing (down to  $\frac{1}{5}$  mm or less), has been fitted into a calibrated illuminator. A device of this kind has been made for the Westinghouse tests, and one is also kept at the Naval Observatory for testing the thin-film tubes manufactured for us by the Radio Corporation of America (Lancaster, Pennsylvania, Works) and by the Farnsworth Corporation.

Efforts to make the necessary uniform films needed for protection of the photoelectric surface from the vapors of the photographic plate, films about 400 angstroms thick but free from pinholes, have met only occasionally with success. The RCA group under Dr. R. G. Stoudenheimer and Mr. J. C. Moor at Lancaster has done considerable experimentation on this problem on its own funds, and likewise the Farnsworth group under Dr. S. F. Essig, with some financial support from our Committee. By far the largest number of films (many hundreds) have been made with numerous variations of technique by immediate members of the Committee group, particularly by Mr. Kent Ford, Jr., of the Department of Terrestrial Magnetism, now working with a grant from the Joint Committee at the University of Virginia. Another long series of experiments on thin films was carried out for our benefit by Dr. Georg Hass, of the Engineers Laboratory, Fort Belvoir, Virginia. These experiments con-

firmed the importance of using partly organic films, as practiced by the other three laboratories; inorganic films, such as titanium dioxide, aluminum oxide, and silicon monoxide, prove too brittle for handling and for bake-out. (All photoelectric tubes must be evacuated and baked at 300° C or higher.)

Experiments with RCA and Farnsworth tubes during the year showed that suitable precautions will eliminate the difficulties that arise from "dark current" and are due to field emission when the required high voltages are applied to the tubes. This was an important and very necessary step.

The Farnsworth Corporation still has funds for the construction of eight tubes when suitable thin films can be provided (probably by Mr. Ford), and the RCA group has funds for making several more tubes. The Westinghouse development continues with our close interest and con-

tact, but without subsidy from the Committee, as Westinghouse wishes to carry this project forward independently. There appears to be a strong possibility, provided the uniformity of the multiplier films is as good as initial tests have indicated, that the Westinghouse multiplier will begin to be useful in astronomical work within a few months. If disappointment is met, however, the thin-film tubes still show every expectation of being usable, although fragile and inconvenient. The completely electronic device of Dr. McGee offers a possible advantage of subtraction of the background, to give greater contrast, but the problems of avoiding the introduction of added "noise" or random effects have caused us to postpone further intensive work on the strictly electronic type of device until the Westinghouse image multiplier and the thin-film tubes have had thorough trials.









SEISMIC OBSERVING EQUIPMENT ON A "CASEY JONES" FLAT CAR OF THE YUKON-WHITE PASS RAILROAD BETWEEN SKAGWAY, ALASKA, AND CARCROSS, YUKON TERRITORY

This photograph shows a typical set-up for recording seismic waves from explosions for crustal exploration. In our studies of the earth's crust we have made observations over all the continental United States, northwest Canada, and Alaska. At this site, chosen because there were no highways traversing the mountain chain in this area, we were recording shots being fired under water by the U. S. Coast Guard cutter *Sedge* near Skagway, about 40 miles distant.

## DEPARTMENT OF TERRESTRIAL MAGNETISM

*Washington, District of Columbia*

MERLE A. TUVE, *Director*

The current impressive willingness of many agencies of society to support research efforts, especially those using tax funds, has a tendency to encourage the young research man toward extremes of specialization and to invite the construction of huge equipments for very limited purposes, or even for single experimental determinations. The preservation of an acute faculty for the perception of beautiful and unexpected regularities or processes in nature is indeed difficult when days and nights of sustained effort, involving the supervision and encouragement of relays of technical assistants, are required to bring some great and complex instrument to the limits of its performance.

In government establishments and perhaps in large institutions for technical training these pressures may be legitimate or at least difficult to avoid, but in an institution such as ours, dedicated to research by individuals of high competence and undiminished interest in the new and unexpected, as revealed in the patterns of nature all about us, it has been our mutual agreement that the largest and most unusual examples of the instruments of modern physics should not be installed here. It appears more likely to be fruitful if only modest instruments, for work in relatively fresh areas of research, should be constructed here, to give our individual staff members the intimate contact with the materials for fresh thinking that is vital in research, without the seductive advantages of experimental material from a uniquely powerful equipment which is beyond the reach of others less favored.

That even these "modest" installations are impressive in terms of cost and effort is a reflection of the great extremes of smallness or of distance or of low intensity or high complexity that are now reached

by modern physical analysis. But in the work of each individual here it is our effort, by frequent vigorous discussion and mutual counsel, to keep the emphasis and satisfactions in the realm of living with ideas, and in the privilege of thoughtful examination and quiet contemplation, not in a large output of facts and measurements.

There is a temptation in physics to build apparatus of tremendous size, almost, it often seems, before the questions to be answered are clearly formulated, as a quick approach to distinction in research, or at least to some temporary degree of unusualness. This "large scale" approach seems to be more or less the accepted pattern for physicists nowadays, in countries the world over. During the war the physicist became a technical man; his goals were not scientific but technical, in that he sought to devise ways of doing practical things, and correspondingly his curiosity and the understanding of natural phenomena became largely secondary matters. At the same time, problems of cost moved out of focus; the goals were war goals supported by the entire fabric of society, through military or military-industrial allotments, and costs became unimportant.

At the end of the war this technical pattern was continued with a high level of support for physicists, and in the United States it was incorporated and accepted to a surprising extent as one of the regular activities of our universities and engineering schools. Many physicists continued to have technical rather than scientific interests, to a large degree. At the same time the notion that faster progress can be made in "research and development" if ample funds are provided was extended to fields primarily of academic interest, with government funds supplied chiefly by military



agencies. This evolution was welcomed by our academic institutions as the only means quickly available for the expansion of their services to the general community.

There is, of course, real truth in the idea that adequate support increases both the research activity and certain types of research output, chiefly, perhaps, research involving elaborate preparation or great precision, or requiring large numbers of slightly varied measurements all of one kind. Ample funds are much less significant in the enhancement of research that requires the gestation of fresh ideas, or calls for the curious probing of minor or common facets of the natural world around us—realities that often prove so unexpected and surprising on detailed examination and thoughtful study.

The staff members of our Department have quietly faced these problems of size and emphasis in their personal activities as physicists. In their assessment of the situation, one of the major advantages conferred by Mr. Carnegie's endowment and preserved by the Trustees and officers for the staff has been the freedom to follow ideas without being harnessed to large equipments or large programs, except by their own personal choice. Any quick enumeration of the active specialties in physics today, however, from nuclear physics to astrophysics, shows that special equipment of various kinds is essential for any experimental research or measurement, and even the cost of small equipment plus allowances for assistants in many research institutions today may add up to sums well in excess of the professional salary budgets for a period of some years.

The Department's activities in radio astronomy have included measurements of the radio noise emitted by the sun, the planet Jupiter, the so-called radio stars, and hydrogen gas of our Galaxy. Last year's discovery of radio noise bursts from Jupiter was followed up this year by observations of the time variation and polarization of the noise. Definite periodicities were found in the occurrence of noise

bursts, which were correlated with the rotational period of the nonequatorial regions of the planet. There are several "active regions" on the planet, the most active of which coincided in longitude with the Great Red Spot. Most of the noise bursts from these regions appear to be circularly polarized, a fact which suggests the existence of magnetic fields in the regions where the radio noise is produced.

In the other programs of radio astronomy it has become apparent that higher angular resolution is now required. For significant solar observations antennas are needed which can discriminate between different active regions on the sun, so that sources of radio noise can be associated with optical features. An antenna of sufficient resolution to separate two sources of radio noise one-tenth of the solar diameter apart has been designed and is now under construction. High resolution is also needed in the study of radio stars. The dozen or so radio sources which are at present identified optically are so varied in nature (of the three strongest, one is an old exploded star, one is a wisp of hot gas, and the third is a collision between two galactic systems each containing billions of stars) that systematic features are hard to find. If the number of optical identifications could be increased to a hundred, perhaps several sources of each kind could be found and their properties studied. Precision interferometers have therefore been designed which should have the accuracy (one minute of arc) needed for optical identification.

Additional contours showing the distribution of hydrogen gas across the galactic plane have been obtained using the present 24-foot reflector to observe the 21-cm radio emission of hydrogen. The possibilities of future measurements with the present reflector are quite limited, however, owing to its low resolution. To obtain the higher resolution needed to observe the detailed structure of the hydrogen clouds, a 60-foot reflector has been designed and the engineering drawings are now



finished. The general design features of this reflector would be suitable for a larger structure; thus it might serve as a prototype for the 140-foot reflector to be erected at the National Radio Astronomy Observatory under the auspices of the National Science Foundation.

In the summer of 1955 the exploration of the continental crust was extended into Alaska and the Yukon Territory. It was found that, as on the Colorado plateau, great topographic heights do not mean that the earth's crust is thick. The variations in crustal thickness which we do find are not simply related to the land height. Continental crustal structure is therefore macroscopically more complex than we had believed.

Rock magnetism studies were continued in a co-operative project with the Bernard Price Institute for Geophysical Research at Johannesburg, South Africa. Efforts were made to confirm the earlier tentative conclusion, reached by studies in the southwestern United States and England, that there had been a sizable shift of the magnetic axis within the past 150 million years. The observations were disappointing because of their inconsistencies, but progress was made in the problem of learning the conditions under which a primary magnetization can survive under the influences of time and natural processes acting in the crust.

Methods of measuring mineral ages using the radiogenic isotopes of argon, strontium, and lead in suitable minerals of igneous intrusives achieved a significant milestone this year. It has now been demonstrated that rubidium-strontium and potassium-argon ages of micas not only are consistent, as had been learned in previous years, but also can now be made absolute ages. The latter result stems from the determination of potassium-argon, rubidium-strontium, and uranium-lead ages of micas and uraninites of a number of igneous rocks of different age.

Previous reports have mentioned the common occurrence of disagreement be-

tween the ages calculated for uranium minerals using the decay of  $U^{238}$  to  $Pb^{206}$  and that of  $U^{235}$  to  $Pb^{207}$ . The reason for this discordance is that the concentrations of uranium and lead in the mineral have to be altered by geologic events after the formation of the mineral. During the past year a method has been developed at the Department for using these discordant ages to deduce in a simple way the probable history of a group of uranium minerals subjected to the same sequence of events. This history includes the time of formation of the minerals and the time or times of their subsequent alteration.

The natural result of the confidence in these new methods of obtaining absolute ages will be the application of these methods to several types of geological problems. One such, which is relatively simple but nonetheless intriguing, is that of examining the gap between meteorite ages, measured at the University of Chicago, which appear to agree at around 4.5 billion years, and the ages of the oldest terrestrial rocks. Intrusive rocks as old as 2.6 billion years have been found in Africa, North America, and Australia. The sedimentary formations which they intrude are still older, and how much older may be learned from the analysis of mica from ancient boulders in these formations. For another problem, micas from the well studied pre-Cambrian areas will be analyzed to learn both the time encompassed by the different formations in this largest span of geologic time and the age relations between pre-Cambrian formations for which no direct field evidence is available.

In nuclear physics, work has continued on the study of low-lying nuclear energy levels by the method of Coulomb excitation. Further evidence has been accumulated in support of the Bohr-Mottelson unified model of the nucleus, which has been successful in accounting for a wide range of nuclear phenomena. The analysis of the laboratory's alpha-alpha scattering data has now been completed and is in agreement with the analysis of recent data

taken at higher energies in other laboratories. It is interesting that the interaction between two rather complex helium nuclei can be represented by a rather simple potential having an outer attractive region and a central repulsive region.

The biophysics work of the Department shows that an attempt to explain some of the complexities exhibited by biological material in terms of forces acting on molecules can provide a stimulating challenge to physicists. In particular, the mechanisms by which small molecules are selected and linked together in the proper order to form the macromolecules of growing organisms are very obscure. Work of the past year has shown that the small molecules are first accumulated within the cells and subsequently linked together by a very rapid reaction. The accumulation process is not sufficiently selective to account for the precise ordering within the macromolecules, as certain similar amino acids can interfere with each other during accumulation. Furthermore, this concentration of small molecules within cells depends upon the osmotic pressure in a way which cannot be interpreted in terms of present cellular models. It appears that the role of internal structures of the cells may be very important, and the properties of these structures therefore require detailed investigation.

It is hoped that the mutual guiding of our activities here, as a company of scholars sharing joys and puzzles with one another, may be visible to some degree in this annual report, and may evoke some sympathetic response to the range of our research interests as physicists, which reach from radio astronomy to the physical chemistry of some of the basic life processes.

DR. JOHN A. FLEMING, 1877-1956

We announce with regret the death of Dr. John Adam Fleming, retired director

of the Department, at San Mateo, California, on July 29, 1956, at the age of 79. He was with the Department from 1904 to 1946, serving successively as chief magnetician, chief of the observatory division and of the magnetic survey division, assistant director, acting director, and director (1935-1946). After retirement he was Adviser in International Scientific Relations to the Institution.

Dr. Fleming had a great capacity for giving his vigorous support to the ideas and interests of his colleagues, especially the younger men of the staff. From the early days of the Department's sea and land surveys, through the hectic days of World War II, his best efforts were given to activities which were, in his judgment, likely to be of far-reaching importance. As a result a number of important scientific discoveries were made with his encouragement and support, including the fundamental studies by Dr. S. J. Barnett in 1920-1921 on the gyromagnetic ratio for electrons in a conductor, the Breit-Tuве radio echo experiments on the ionosphere, which provided the basis for radar, the world-wide cosmic-ray investigations using Compton-Bennett meters, and some of the earliest work in the United States on nuclear physics using high-voltage accelerators. For a decade before World War I he guided the world magnetic survey on land and sea by the Institution, and participated in the designs of the improved instruments used in this survey. He edited the *Journal of Terrestrial Magnetism and Atmospheric Electricity*, now the *Journal of Geophysical Research*. He was the president of several scientific societies here and abroad and was a member of the National Academy of Sciences. He was the recipient of the William Bowie medal of the American Geophysical Union and the Charles Chree medal of the Physical Society of London.

## EXPERIMENTAL GEOPHYSICS

## RADIO ASTRONOMY

B. F. BURKE, W. C. ERICKSON, J. W. FIOR,  
K. L. FRANKLIN, AND H. W. WELLS

## MILLS CROSS

Observations with the 22 mc/sec Mills Cross, described in previous reports, were continued, primarily with a view toward critically evaluating the potentialities of this system. Selected areas of the sky were observed, the most extensive coverage being of the region between declinations  $+35^\circ$  and  $+43^\circ$ . It became clear from these studies that only a few of the principal radio sources could be observed with a large signal-to-noise ratio. A considerable number of sources, barely visible above the noise, appeared to be present, but the patterns obtained at adjacent declinations, less than a beam width apart, were not always consistent. This inconsistency raised the suspicion that some of the weak sources were not real, but were spurious responses due to a strong source such as Cygnus A or Cassiopeia A. A check upon the reality of these weak sources appeared desirable, a simple measure being to construct an auxiliary array in an adjacent field, to be used in combination with one or both arms of the cross as an interferometer. While this would discriminate against sources of large angular size, the comparison of the interferometer trace with the records from the cross should provide a useful check on the reality of weak sources. A 64-dipole square array has been constructed 2200 feet east of the center of the cross. In addition to its use as an interferometer, the square array will be used for surveying broad features of the galactic background, and should provide data on the reality of the "galactic corona," the roughly spherical, extended region of radio emission in which our Galaxy appears to be embedded.

An interesting result obtained from the survey of this region was the detection of the well known source Cygnus X, but in absorption instead of emission. The ob-

served brightness profile is almost a mirror image of the Ohio State University 250 mc/sec observations. It has been suspected that this source is an extensive, dense region of ionized hydrogen obscured from optical observation by dust clouds. The 22 mc/sec observations lend support to this interpretation, since such an H II region would absorb the general background radiation coming from beyond the source. The surface brightness of the source (at 22 mc/sec) would be in the vicinity of 10,000, a considerably lower brightness than the neighboring galactic background.

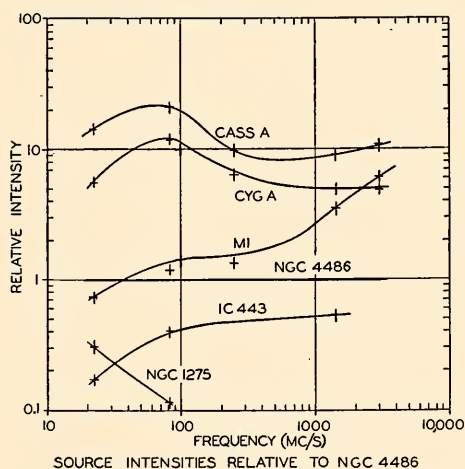


FIG. 1. Relative intensities of several strong radio sources, the Virgo source taken as unity; 22.2 mc/sec points are from cross measurements, and the highest frequency points are from various workers.

Absolute calibration of the cross must await an accurate determination of the flux of one of the principal sources, but relative intensities have been obtained for several of the brighter sources. Figure 1 compares these intensities with the relative fluxes observed at other frequencies, where the Virgo source, M 87, has been taken to be unity. A difference in behavior of the relative spectra can be noticed, the galactic sources M 1 and IC 443 dropping in relative intensity quite markedly at 22 mc/sec,



compared to the extragalactic sources and the Cassiopeia source.

### JUPITER

After the initial discovery in early 1955 that Jupiter was a source of radio-frequency radiation, plans were made for a new instrument that would observe Jupiter over a longer interval of time each day than was feasible with the Mills Cross. During the summer and fall of 1955, a new instrument was designed and assembled at the River Road site.

The antenna initially consisted of two arrays separated by 20 wavelengths along an east-west line. Each array contained eight half-wave dipoles, separated from each other by a little more than a half wavelength, along the north-south direction. The individual dipoles in each array were oriented east-west. The entire antenna was designed for operation at a frequency of 22.2 mc/sec (13.5 m). This system allowed Jupiter to be observed for approximately seven hours each day. The antenna and receiver, used as a lobe-switching interferometer, were put in operation October 31, 1955, and a series of observations was begun.

The results of this phase of the work indicate that Jupiter was not as active during the 1955-1956 opposition as during the previous season, when it was discovered. None of the activity is easily correlated with any feature rotating with the rate pertaining to the equatorial regions (System I,  $9^h 50^m$ ). The data show, however, that there were four regions of interest on the planet all better correlated with the period of the nonequatorial zones (System II,  $9^h 55^m$ ).

Figure 2 represents a portion of the data in the following way: A horizontal line in the diagram denotes the longitude of the central meridian of Jupiter's disk during the time radio noise was detected. The thickness of the line is an indication of the intensity of the noise. The longitude system used is that for the nonequatorial regions.

The most active area has been near longitudes  $300^\circ$ - $320^\circ$  (System II), with a rotation period of  $9^h 55^m 33^s \pm 8^s$ . A prominent surface feature near these longitudes, and with a slightly longer rotation period, is the Great Red Spot. An interesting aspect of the radio emission from this area is the sharp polar diagram, activity being confined to a  $60^\circ$  rotation of the planet. A second active region was around a longitude of  $30^\circ$ - $40^\circ$ , and a third, possibly multiple-active, area, has run from  $140^\circ$  to  $260^\circ$ . The fourth area of interest has been that from  $70^\circ$  to  $120^\circ$ , from which we have detected no activity at 22.2 mc/sec.

The radio data have been communicated to Dr. A. F. O'D. Alexander, Director of the Jupiter Section of the British Astronomical Association. Dr. Alexander has been actively interested in these phenomena, and has encouraged the members of the Jupiter Section in careful, continued observations of visual features of Jupiter's apparent surface. E. J. Reese, a member of the B. A. A., from Pennsylvania, has supplied several positions of visual features to be compared with the locations of radio features. These are shown in figure 2 as lines labeled *FA*, *BC*, *DE*. These features are elongated white regions located in a band just to the south of that containing the Red Spot (*RS* in fig. 2). The region labeled *STrD* is the South Tropical Zone Disturbance, a variable feature located in a band just north of that of the Red Spot. No clear correlations can be made with the large visual regions, with the exception of the Red Spot, noted above.

Lowell Observatory at Flagstaff, Arizona, has obtained approximately six dozen photographs of Jupiter, in a co-operative optical-radio program of observation. Unfortunately, none was exposed during the occurrence of a radio event observed at the Department of Terrestrial Magnetism.

Dr. R. S. Richardson, of the Mount Wilson and Palomar Observatories, has also contributed to this co-operative program. On one occasion, he was observing Jupiter



with the 60-inch reflector on Mount Wilson during a radio event, but his material has not yet been fully discussed.

H. W. Wells made available the records obtained with his interferometers located

other two frequencies available. When there were simultaneous occurrences on two or three frequencies, the individual bursts comprising the event were not correlated in sequence or time between the

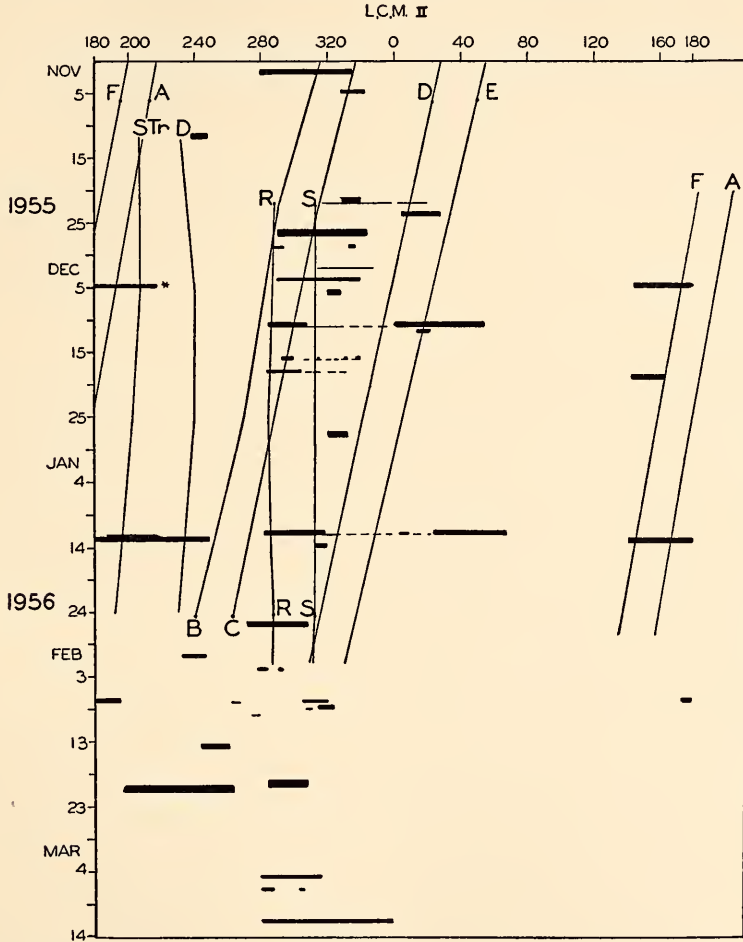


FIG. 2. Occurrence of radio noise from Jupiter during 1955-1956. Longitude of central meridian in System II is indicated for the observed radio noise bursts, together with the location of a few prominent visual features.

at Derwood operating at 18.5 mc/sec and 26.75 mc/sec. These records were successfully examined for observations of Jupiter events. It appeared that the radio radiation from Jupiter has interesting spectral properties. Often, an event recorded at one frequency was not observed at the

frequencies used. This property has also been observed by K. Bowles and R. Gallet in Boulder, Colorado, but correspondence with C. A. Shain in Australia reveals that there is high correlation, burst for burst, between events recorded at frequencies separated by less than 100 kc/sec, near

19.6 mc/sec. Although the individual events may occur at different times on the several frequencies, the sources of radiation on Jupiter show essentially the same distribution on the planet as has been mentioned in connection with the 22.2 mc/sec data. See figure 3. Qualitative spectral information could also be derived by comparing amplitudes relative to Cassiopeia A at different frequencies. The bursts, in general, increase markedly in intensity with decreasing frequency.

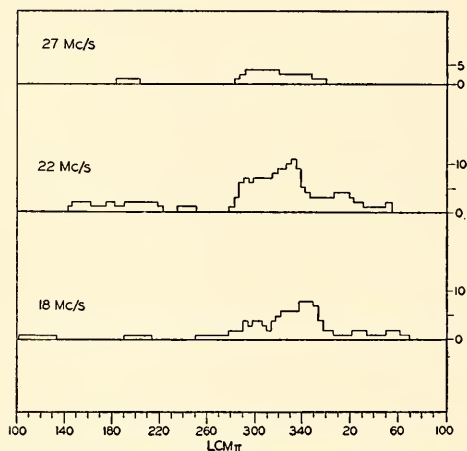


FIG. 3. Longitude of central meridian (System II) for noise bursts observed from Jupiter at several frequencies.

In January 1956, a third array, consisting of eight dipoles arranged in line in the north-south direction, was added to the interferometer, 20 wavelengths from one of the previously established arrays of east-west oriented dipoles. This additional array, used in connection with the nearest of the other two, made a system capable of detecting the presence of circularly polarized radiation. The original pair was sensitive to the east-west linear component of any polarization. With this equipment, it was soon found that the radio-frequency radiation from Jupiter was usually circularly polarized. Events of nearly 100 per cent polarization have been observed, usually corresponding to the right-hand sense

of rotation. Occasional events of weak or mixed senses of polarization have also been recorded. If a source of radio emission is assumed to be below a Jupiter ionosphere, and if a cutoff frequency for the extraordinary ray is assumed to be 22.2 mc/sec, it is possible to set a lower limit to a magnetic field on Jupiter of about 4 gauss. If the cutoff frequency is higher, the magnetic field is increased correspondingly.

To test this possibility, an antenna, basically similar to that described, was erected at Derwood to operate at 26.75 mc/sec. Previous experience indicates that a small number of events should be observed at this frequency, but no clear-cut examples have yet been observed.

If the indicated magnetic field on this model is general, and oriented in the same manner as that of the earth and of the sun, events exhibiting right-hand circularly polarized radiation should originate in Jupiter's southern hemisphere, left-handed in the northern. If there are local magnetic fields near the sources, however, no clear statement can be made now about the locations of the sources.

#### OBSERVATIONS AT 200 MEGACYCLES PER SECOND

The realization that the meter wavelength radiation from the sun originates largely in the corona has prompted several laboratories to measure the amount and distribution of the radio energy from the sun as a method of studying the corona. The difficulty encountered in the optical observations of the corona—the large background of light from the photosphere—is not experienced in the analogous radio studies, since the radio radiation originating at the surface of the sun cannot escape through the ionized gases of the chromosphere.

At wavelengths in the range 10 to 60 cm, measurements made in many countries agree quite well as to the brightness and the distribution of brightness of the

quiet sun, and the measured results in turn agree roughly with the predicted radiation from the corona. At longer wavelengths the agreement between observers and between observations and theory has been poorer, owing partly to two difficulties in the observations occasioned by the longer wavelength. The first difficulty is that the required angular resolution is harder to obtain and must be done interferometrically. The second, and related, difficulty is that bright areas on the sun associated with temporary regions of activity are hard to detect and separate out when using interferometers.

At Derwood we have tried to circumvent these difficulties and obtain a picture of the sun at a wavelength of 145 cm. Two methods were used to avoid the effect of bright areas. First, the measurements were made in 1954 and 1955 when the sun was mostly inactive and only a few bright areas occurred. Second, the position of the radio sun was repeatedly measured. Any bright area not near the center of the disk of the sun would shift the apparent position of the sun and hence its presence would be noted.

Two interferometers were used, one arranged along an east-west line and one on a north-south line. The sun was thus analyzed in a stripwise fashion with two mutually perpendicular sets of strips. The distribution of brightness derived from such an analysis is not unique, but brightness contours can be found that are consistent with the measurements.

The sun at 145 cm appears larger than the optical sun, according to these measurements, and is elliptical rather than circular. Furthermore, the brightest part of the disk is not the center but a ring around the center. All these features are in substantial agreement with theoretical predictions based on a simple model of the corona.

*New interferometer.* With the sun growing more active as the sunspot cycle progresses, measurements of the sun with conventional two-element interferometers

will become more difficult, since there will be an increasing number of bright areas to contend with. A new interferometer has been designed and put under construction which should avoid some of the difficulties encountered in interpreting the usual interferometer records. It is a multi-element interferometer, with 48 identical elements arranged on a line 3000 feet long. The beam width will be 3 minutes of arc. This very narrow beam should allow bright areas to be recognized and separated from the quiet-sun background. The new antenna will be used at a wavelength of about 1 meter.

#### LOW-FREQUENCY RADIO ASTRONOMY

Preliminary mention of successful efforts to identify some of the major discrete "radio stars" at frequencies as low as 12.5 mc was made in the preceding annual report (see Year Book No. 54, 1954-1955, pp. 47-48). Some early results were included in a report on the Department's work at meetings in Manchester and Dublin during the summer of 1955. It was later established that the calibration methods originally used were inadequate for purposes of absolute measurement in view of saturation effects in the receiver. Subsequent instrumental improvements have made it possible to apply calibrations without disconnecting antennas from the receiver. Hence, the application of calibration signals, under actual operating conditions with antennas exposed to all sky noises, has eliminated saturation errors. It is estimated that flux measurements originally reported may have to be increased by a factor of about 2, and work is actively under way on the redetermination of intensities of the principal radio stars at low frequencies.

It is already apparent, however, that it may not be possible to repeat observations in the 12-15 mc range as a result of the new solar cycle. The effect of increased solar activity has been very apparent during the past few months. At the present time we

have a few hours per night of useful recording through the ionospheric "window" at 18.5 mc. Attempts to observe at 15.5 mc, however, have been entirely fruitless, with no periods even in the presunrise hours when the ionospheric "window" would be open. Perhaps it should be explained what is meant by the ionospheric window. Although signals from radio stars reasonably close to the zenith would penetrate through the ionosphere, the only condition under which it is possible for us to observe these radio sources is in the absence of interfering signals reflected from terrestrial sources by the ionosphere. The maximum usable frequency for oblique incidence at maximum horizontal range must therefore be lower than our observing frequency.

#### FLUX MEASUREMENTS (COLOR INDEX)

Requirements for absolute measurements include (1) knowledge of effective antenna aperture, (2) precise measurements of all losses in transmission lines or associated components, (3) a reliable source for calibration, and (4) elimination of non-linear or "saturation" effects. The determination of antenna aperture requires knowledge of the antenna polar diagram and the efficiency of the reflecting surface. Our absolute measurements are being made with the simplest of antennas—dipoles—mounted over a large grid of reflecting wires. For measurement of antenna efficiency, a reference dipole antenna has been installed over a large, flat continuous plate of sheet metal which is considered to be perfectly conducting. A method has been devised to intercompare, using radiometer techniques, the relative powers of the reference antenna and the actual antenna. It has been determined that the antennas in use at 26.75 mc are between 90 and 95 per cent efficient.

Comprehensive tests have been conducted to intercompare the noise power of conventional noise diodes with that of thermal sources at frequencies below 30 mc. Over an equivalent temperature range

from 0 to 200° C, the agreement is excellent, probably better than 95 per cent.

Saturation effects were mentioned earlier. They have been virtually eliminated by the use of "magic T's" and signal-splitters permitting calibrations to be applied simultaneously with antennas in operating condition.

During the spring and early summer of 1956, it was possible to determine flux measurements for Virgo at 26.75 and 18.5 mc, and for Taurus A at 26.75 mc. The values are indicated in table 1.

TABLE 1

SOURCE	FREQUENCY	
	26.75 mc	18.5 mc
Virgo .....	50 *	150 *
Taurus A .....	50 *	...

\* Units of  $10^{-24}$  watt per square meter per cycle per second.

Relative measurements of the ratio of Cygnus A to Cassiopeia A have been made at 18.5 and at 26.75 mc. These observations required the use of more elaborate antenna systems such as three- or four-element col-linear arrays in order to resolve between the two sources. The ratio of Cygnus A to Cassiopeia A at 26.75 mc is very close to 0.6, while the ratio at 18.5 mc is approximately 0.5 (see fig. 4). Of the two measurements, more confidence is attached to the one at 26.75 mc, since it represents the average of many more observations than the one at 18.5 mc. The difference is large enough, however, to justify a conjecture that the apparent reduction in intensity of Cygnus A at 18.5 mc may be related to interstellar absorption in view of its extreme range (34 megaparsecs) compared with the range of Cassiopeia A (500 parsecs).

#### HYDROGEN-LINE OBSERVATIONS

H. E. TATEL, M. A. TUVE, E. T. ECKLUND, AND  
K. L. FRANKLIN

The continuing development of the hydrogen-line radio observations at many ob-



servatories has greatly enhanced our knowledge of the galactic structure. Measurements at other observatories have already been made on other galaxies.

During much of the report year, the equipment for observing the 21-cm line radiation from neutral hydrogen has been undergoing improvement modification preparatory to conversion into a multi-channel system. This program has not yet been completed.

The chief observational program, the survey of hydrogen along galactic meridians, has continued. Satisfactory material now exists for galactic longitudes  $50^\circ$ ,  $60^\circ$ ,  $80^\circ$ ,  $90^\circ$ ,  $110^\circ$ ,  $200^\circ$  between galactic latitudes of  $\pm 20^\circ$  as well as similar, but less

stellar data into the form used to present the radio data.

A major difficulty with the conversion of the hydrogen observations into true spatial distribution is that a theory relating distance to velocity, based on the concept of a rotating galaxy, must be relied on. Although the idea is entirely satisfactory, the mathematics rests on certain assumptions valid only for regions near the sun. Many of the regions observed in the radiation of neutral hydrogen fall far outside the range of confidence in the theory; no other satisfactory means yet exist, however, for assigning such distances to the observed hydrogen. The comparison of observed hydrogen data with those for bright, blue

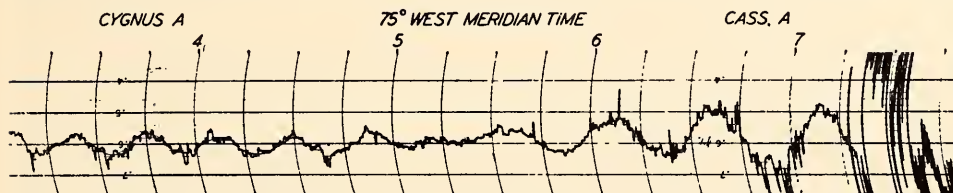
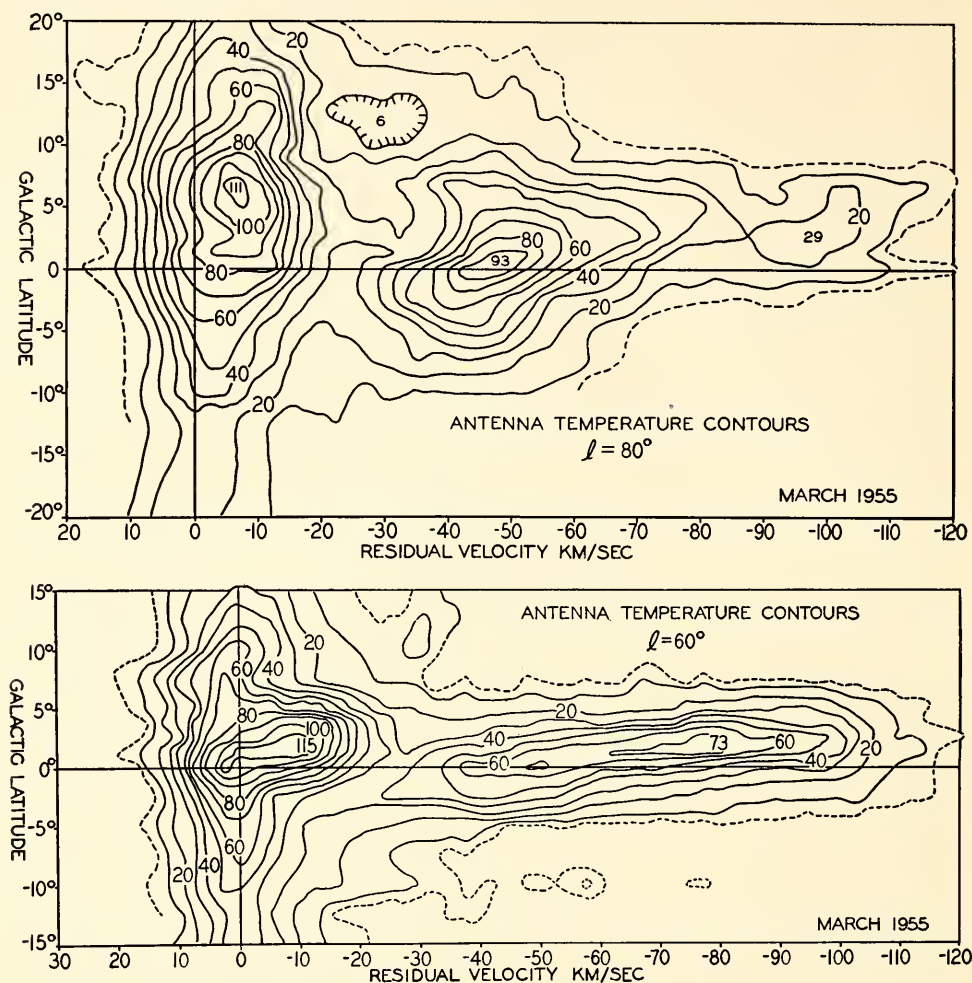


FIG. 4. Interferometer record of Cygnus A and Cassiopeia A at 18.5 mc/sec, May 27, 1956, Derwood, Maryland (the ratio of Cassiopeia A to Cygnus A is approximately 2 to 1).

complete, data for several other meridians (see figs. 5 and 6).

The observed data comprise a figure for antenna temperature (related to the density and temperature of hydrogen gas) produced by the hydrogen radiation received from a certain direction (galactic longitude and latitude) at a particular frequency of the radiation (related to the velocity in the line of sight of the aggregations of hydrogen gas). One of the most straightforward ways of presenting these data is to plot in these dimensions the antenna temperature as a function of velocity and galactic latitude, at a given galactic longitude, and to represent the antenna temperature as contours of equal values on the latitude-velocity plane. In order to compare other astronomical information similar to that observed for hydrogen, it is a simple but tedious task to convert the

stars (spectral types O and B) indicates that, on any theory relating velocity with distance, the distance to the concentrations of stars in the direction of  $\tau=90^\circ$ , for instance, may be less than that to the peak of the hydrogen concentration by as much as half the thickness of a spiral arm (about 500 parsecs). Similar conclusions may be drawn at other locations. All the stellar data have been obtained from R. E. Wilson's *General Catalogue of Stellar Radial Velocities* (Carnegie Inst. Wash. Pub. 601, 1953). The fact must be noted, however, that a radio observation of hydrogen gives information on practically all the hydrogen in the line of sight, whereas similar information may not exist for all O and B stars in the same volume of space. To obtain stellar information as complete as that obtained for neutral hydrogen, much more optical work may be necessary, especially



FIGS. 5 and 6. Measurements of radiation from atomic hydrogen in planes which cut the Milky Way at galactic longitudes  $80^\circ$  and  $60^\circ$ . The tendency for hydrogen clouds to be distributed in roughly spiral "arms" is evident. Similar measurements have been made for various other planes perpendicular to the galactic plane.

the observation of radial velocities of the fainter stars.

#### THE UPPER ATMOSPHERE

B. F. BURKE, W. C. ERICKSON, J. W. FIROR,  
AND H. W. WELLS

Interest has continued in the characteristics of radio star scintillations. For this investigation the 26.75-mc interferometer at Derwood has been of special service. Observations over the past year have partly

confirmed the earlier reported over-all characteristics of scintillations with maximum activity close to local midnight. Scintillations are often more pronounced for sources overhead or to the north, however, than for sources toward the equator, regardless of local time. Frequent recordings have been made of the slow and fast types of scintillations as well as the abrupt transition from quiet to scintillation. Preparations are being made for

measurement of size and movement of the ionospheric irregularities causing scintillations. The experiment will initially consist of two stations along an east-west base line, to be supplemented later by a third station in order to obtain a more complete description of the phenomena.

A joint experiment with the University of Virginia is under development in order to learn more about the large-scale atmospheric motions involved in the abrupt transition from quiet to scintillation conditions. After the University's new observation network for the International Geophysical Year is established, we hope to obtain some useful information on mass movements in the upper atmosphere related to precise timing of the occurrence of the abrupt transition at stations separated by approximately 100 miles.

In preparation for a report for presentation at the AGARD Symposium on Polar Atmosphere, Oslo, Norway, July 1956, additional analyses of the observations made during the interval 1950-1953 for the determination of dynamic characteristics of the ionosphere were conducted. In particular, new facts about the apparent downward component of movement were established. Most of the traveling ionospheric disturbances appear to move into the east and have a wave front inclined approximately  $45^\circ$ . The effects observed at spaced stations may be adequately explained by an inclined front moving horizontally or downward through the atmosphere. Similarly, any blending of the two types of movement would produce the same overall effect.

#### FACILITIES AND INSTRUMENTS

The principal instruments in use at Derwood (15 miles north of Washington) have been mentioned separately in the body of this report. To summarize, however, interferometers are in continuous or intermittent operation at frequencies of approximately 200, 26.75, 18.5, and 15.5 mc. Most of them are arranged for a maximum degree of flexibility, permitting different

spacing of antennas, different types of antennas, and varying recorder characteristics, depending on the nature of the experimental application. At River Road (21 miles west-northwest of Washington), the principal instrument is the Mills Cross at 22.75 mc. Other supplementary instruments for Jupiter patrols or polarization experiments have already been mentioned. The multifrequency ionosphere recorder at Derwood has been used on an occasional basis principally for purposes of demonstration or for a quick look at ionospheric conditions as a supplement to the scintillation or other radio-astronomy investigations.

### THE EARTH'S CRUST

#### SEISMIC STUDIES

H. E. TATEL AND M. A. TUVE

During the past several years we have reported upon the gradual progress of our seismic explorations of the earth's crust. Our primary concern has been to explore the structure of the continent, making measurements and comparisons of different regions and hoping later to be able to contrast the over-all continental crustal structure with the structure of ocean basins, as determined by others.

Gravity measurements are sensitive to mass differences underlying a region and are a complement to seismic measurements. Thus, with depth from seismic measurement and mass differences from gravity surveys, a more detailed knowledge of crustal properties is obtained, and better tests of structural hypothesis are possible. Such a basic question as whether or not a lesser crustal thickness is accompanied by an excess in gravity, because the underlying dense rock is then nearer to the gravity meter, becomes open to test. An answer to such a question is a direct approach to the problem of the relative prevalence of local stress relief and hydrostatic equilibrium as contrasted to the support of local structures by stressed crustal material. During the summer of 1955 we made both seismic and gravity measure-



ments in Alaska and the Yukon Territory. The results were different in the various localities, showing in some places a direct correlation between gravity and seismic measurements, in the sense of normal crustal structure and small gravity anomalies, and in other places showing evidence of a thicker crust with unexpected positive gravity anomalies.

The expedition during 1955 was more extensive than previous ones in that we traveled very great distances. We drove our six field-equipped cars from Washington, D. C., over the Alaska highway and back. Each car was driven a total of more than 13,000 miles in the two months we were away; some of the observing locations were more than 5300 miles distant from home base by the shortest route.

The suitability of Alaska and the Yukon Territory as a field of exploration is not determined by the particular properties there of the earth's deep crust, but by the superficial geography. In these areas there are high mountains close to the sea and penetrated by navigable fiords with little commerce or fishing, thus making explosions feasible. With the assistance of the staff of the Office of Naval Research, arrangements were made to have surplus depth charges sent to Alaska. The staff of the U. S. Coast Guard assigned a Coast Guard cutter to set off these explosives for us. In spite of adverse conditions such as storms, disabled ships, and extra emergency tasks, the men of the Coast Guard carried through their voluntary commitment to us with admirable effectiveness and made possible the measurements reported here.

An expedition such as this is difficult to arrange, as it requires the assistance of people whose work and training are in fields quite different from geophysical research. Thus, once an expedition is arranged, it is important for us to make the most of the opportunity presented by collecting as many measurements as possible. For this expedition we built two additional

complete seismic field outfits, including two-way radios. Our very limited personnel for seismic studies was doubled by additions from our colleagues in biophysics, two from the staff (R. Britten and R. B. Roberts), and one graduate student (T. W. Tuve), who gave up their summer activities to join us on the expedition. We were thus enabled to double the number of seismograms we recorded from the fixed number (21) of available explosions. We had six fully equipped field vehicles in use, and we used every existing road in Alaska and the Yukon Territory for travel and measurement (fig. 7).

The first series of explosions was set off at the foot of a glacier in isolated College Fiord (fig. 7), about 100 miles east of Anchorage. Our parties were divided into three groups of two, the members of each pair working within a few miles of each other for safety, one pair along the Kenai Peninsula, another to the east near Valdez, and the third beyond Gulkana to the northeast. The first explosion was the climax of an eleven-day wait while our explosives were in transit. After a long search was made for the 22 tons of depth charges, which had been lost in shipment between Indiana and Alaska, they were found in storage 60 miles from Seattle. After the first shot, a radio message from the U. S. Coast Guard cutter *Citrus* announced that one more shot would be fired and then the ship would have to rush to Kodiak and from there proceed on an emergency mission. But the U. S. Coast Guard had agreed to set off these charges for the Carnegie Institution, and, despite the shortage of vessels and the great pressure of other work, our charges were transferred to the U. S. Coast Guard cutter *Sedge*, which was sent to College Fiord.

The *Sedge* fired one shot, then radioed to the field parties that a distress call, which of course took precedence over our work, had been received and further shots must be postponed. Shooting was resumed two days later, and we were interrupted





FIG. 7. Map of Alaska and northwest Canada. The circles, centered at the shot points, have radii of 100, 200, and 300 km, respectively. The dots are the observing points where good seismograms were recorded.



only once more during the succeeding days. These experiences made the seismic traces seem great treasures to each observer as they were recorded.

To the southeast along the Kenai Peninsula, we found the normal set of seismic arrivals, including a critical reflection and the compressional wave in the mantle (fig. 8)  $P_n$  at 280 km. The latter was obtained only through the initiative of one of our biophysics colleagues, who rented a motorboat and made measurements across the bay from Homer at the tip of the Kenai Peninsula. The gravity measurements indicated nothing unusual, the crustal thickness being about 35 km.

To the east, along the road from Valdez, early seismic arrivals were observed (fig. 8). They were appreciably earlier than those at similar distances along the Kenai Peninsula to the southwest. In addition, a gravity anomaly was found in the same region. An estimate of the isostatic anomaly over this region was made. It is positive. The

combination of the two sets of data implies that there is a buried lens of high-velocity rock not far below the surface. It must be supported by a lower-density structure beneath it, or by stress in the near-by rock.

The second set of explosions was set off in a fiord near Skagway, Alaska (fig. 7). Measurements were made on the roads and along the narrow-gauge railroad from Skagway to Whitehorse, Yukon Territory. An excellent group of critical and total reflections was observed (fig. 9). Contrary to what might be expected, to the north the crust appears thicker by 4-5 km than to the east, whereas the gravity measurements show a mass deficiency to the east. Although all the implications have not been explored, several are at once evident. The density gradient within the crust may be different or the density gradient in the "mantle" under the crust may be different in the two directions from the shot, or the relation between density and

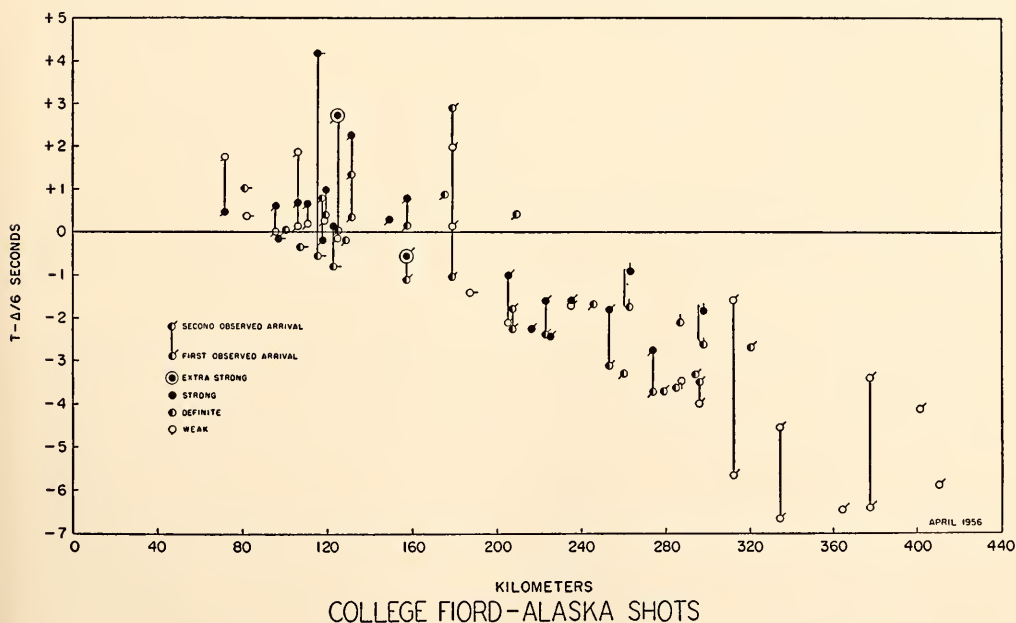


FIG. 8. This is a "reduced time" plot showing the observed arrivals at the various distances from the shot point. The early arrivals in the interval 85-170 km for observations to the east are correlated with a positive gravity anomaly and could be caused by a buried lens of dense material.

seismic velocity may change very rapidly. The significant result will be the determination of the seat of the difference in crustal properties: whether it resides within the crust or beneath the crust in the mantle, or, what is more likely, in both. One clue we do have is from our gravity measurements, which show that throughout the region over which we made our

In the laboratory, further investigations have been carried out on the subject of conversion scattering. Previous field and model work has shown how this phenomenon can account for a large part of the ground motion recorded after the arrival of the compression wave and before the arrival of the direct Rayleigh wave. The question we wished to clarify was

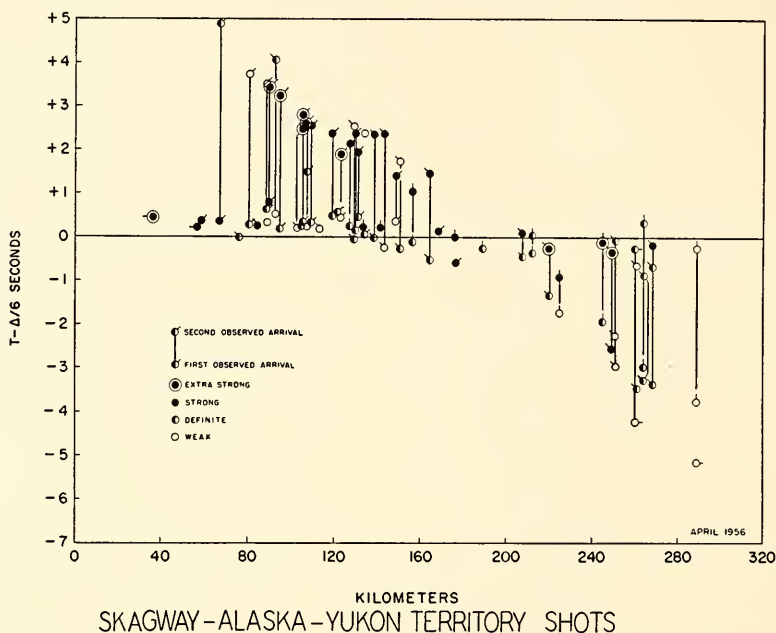


FIG. 9. The set of arrivals shown here indicates a single crustal structure. The pronounced system of second arrivals in the interval 80-160 km is due to a well defined seismic discontinuity at a depth of 32-37 km (uncorrected for velocity change with depth). Mean topographic elevation is about 4000 feet.

seismic measurements there is a widespread positive gravity difference with respect to an isostatic reduction. Such a gravity difference is not unusual in mountain regions. Other observers have found mountains with either positive or negative residuals extending over a considerable area. The inference is that there are deep-seated density differences or stress differences at levels that are considered to be within the mantle. What we found in the mountains near Skagway may be an example of this situation on a small scale.

this: if there is such strong  $P \rightarrow R$  (compression to Rayleigh wave) conversion as shown by the model work, why do we observe so few Rayleigh waves on our multiple seismograms? If there is dispersion due to increase of velocity with depth, it is possible for the converted Rayleigh wave to show a phase velocity that corresponds to the velocity of compressional waves. Nonetheless, some Rayleigh wave groups should be observed in the field, and they are rarely seen. In the model experiments, where conversion was caused



by surface irregularities, Rayleigh waves (before the arrival of the direct Rayleigh waves) were indeed observed. If a sufficient number of small obstacles (holes) are placed just below the surface, however, almost all the direct and converted Rayleigh waves are found to be suppressed in the model seismograms. Hence the answer to the problem is quite simple: in an ordinary field situation there must be such a complete and rapid conversion of the Rayleigh waves by the irregularities at and near the surface that they are rarely seen as a Rayleigh group. This reasoning is borne out in a general way, because, at 100–200 km from an explosion, the Rayleigh wave should be many times the amplitude of the compressional wave (first arrival). Actually, the direct Rayleigh wave is rarely observable as an entity, and in some cases the motions shown on the seismogram have all but disappeared into the background of unrest by the time the Rayleigh wave arrives. Therefore the near-surface part of the crust must be highly inhomogeneous, thus providing an almost continuous set of scattering centers. From the effect on the direct Rayleigh wave the size of these scattering centers must be of the order of a wavelength or less (about  $\frac{1}{2}$  km), and they must extend to a depth of a kilometer or so.

#### ROCK MAGNETISM

J. W. GRAHAM

The problem of tracing the history of the earth's magnetic field at remote geologic times continues to increase in complexity. The experimental program at this Department began in 1938 with the elementary notion that sediments received their initial magnetizations at the time of deposition and retained them to the present day. This assumption has proved adequate in only a fraction of the sediments we have studied, particularly the varved clays. Although some other mechanism following deposition appears to be responsible for the magnetizations reported last

year for the Permian and Triassic sediments of the southwest United States, they nevertheless seem of special interest because from them a magnetic pole location in the vicinity of Korea can be inferred. The same location was previously deduced by others from observations in sediments of approximately the same age in Britain.

This inference that there had been a significant shift of the magnetic axis from another orientation lying outside the limits of variability indicated by younger rocks and classical observations, called for an independent examination in some locality well separated from the other two. South Africa was chosen for its extensive series of sediments, which range in age from the Carboniferous into the Triassic.

Workers at the Bernard Price Institute for Geophysical Research of the University of the Witwatersrand were already engaged in a program of research in rock magnetism, and their enthusiasm for a co-operative research effort was immediate. One of our staff members paid them a two months' visit, during which extensive studies were made in numerous localities of the Union of South Africa.

Unfortunately, the sampling problem proved formidable. Where the sediments are flat-lying, they are poorly exposed, badly weathered and fractured, and extensively invaded by sills of igneous rocks. Exposures are good, and the intrusives lacking only where the sediments have been severely deformed. These difficulties will have to be circumvented by collections made during the sinking of a mine shaft in a favorable area. This program is being continued by the Bernard Price Institute.

Sampling and measuring the magnetizations of the igneous intrusives, the Karroo dolerites, proved easy, but the results were disappointing. No magnetization pattern impressively consistent over a large area was obtained, and there was no suggestion of a confirmation of the previously deduced pole location in Korea. No obviously sound explanations for the internal inconsistencies and the lack of

confirmation of the earlier deductions were adduced during the visit.

Later consideration of the factors that might influence the magnetization process of the dolerite sills led to the recognition of magnetostriction as a parameter of possible importance that should be explored fully. Magnetostriction is recognized as the change of length of a sample when a magnetic field is applied. Conversely, the magnetic moment of a sample having the property of magnetostriction will be changed when the sample is nonuniformly strained. It is known from studies of synthetic compounds, which are similar to the magnetic ingredients of rocks, that subtle changes in composition can greatly influence this property, both in magnitude and also in sign (i.e., whether the sample is made longer or shorter by an applied magnetic field). Exploratory studies on some representative igneous rocks were successful in showing that a directed stress of 3000 pounds per square inch was adequate to change the magnetization of the samples markedly. This stress is equivalent to the loading that would be produced by a column of rock some 2000 feet high.

Customarily one thinks of rocks at depth as being in simple hydrostatic loading. In the case of dolerite sills, however, this usual circumstance is changed by special conditions. Sills originally were injected molten between the bedding planes of a thick sequence of sediments. Characteristically they are thin in comparison with their lateral dimensions. In cooling, the sills shrink, and, below some fairly high temperature when the melt becomes rigid, the shrinkage cannot be accommodated solely by shortening in the vertical direction. As a consequence, in the ideal case, a family of vertical fracture planes develops that divide the sill into an assembly of isolated vertical columns. These now are supporting the overlying blanket of sediments. The steps that lead ultimately to freeing the sample for measurement and unloading the compression will allow magnetostriction to produce in it a mag-

netic moment that is unrelated to the earth's magnetic field.

From the above facts and arguments, the observed inconsistencies in the Karroo dolerite sills may possibly be understood. (They serve well to illustrate the fact that, of all the rocks studied, only a fraction is suitable for paleomagnetic purposes.) Further investigation of the role of magnetostriction in the problems of rock magnetism may prove likewise rewarding, even though its troublesome influence on efforts focused on paleomagnetism cannot be easily eliminated from certain rocks. Fortunately, it apparently can be avoided by the proper selection of samples, as is indicated, for example, by the many consistent results that have been obtained in lavas that cooled on the surface of thin sheets.

An estimate of the place of rock magnetism in the science of geophysics today is perhaps not out of order. Within the past couple of years there have appeared a number of serious papers dealing with the subject of polar wandering, by which is meant a shift of the geographic features of the earth's surface with respect to the axis of spin. Classical geophysical treatments of the type pioneered by Sir George H. Darwin early in this century have been re-examined in the light of our more recent knowledge of the earth and its properties, and the conclusion is reached that, whereas polar wandering was formerly considered impossible, it now seems to some, at least, to be inevitable. These re-examinations were inspired by deductions based on the rock-magnetism studies of the past few years. Even though in both fields of interest there remain uncertainties, it appears hopeful that we are approaching a more accurate understanding of the earth's long-time behavior. Some regionally consistent patterns of magnetization are most simply interpreted on the basis of polar wandering, and theoreticians now grant that wandering is possible. These tentative conclusions are in marked contrast with the strong convictions of the previous several decades.

The many studies now being conducted throughout the world may give some indication whether these notions are correct.

In still another field we face the possibility that studies of rock magnetism will require us to modify our prejudices. The concept of continental drift has been debated for nearly fifty years. During that time an amazing array of details, mainly geological, have been assembled in support of the idea, but geophysicists have been staunch opponents, mainly on the basis that there is no possible way, within our framework of knowledge of the properties of the mantle and crust, for continental sectors to be translated relative to one another. With an adequate number of measurements of rock magnetism in suitable rocks, we may be able to describe quantitatively such movements as may have occurred in various geological epochs. Already, some of the available magnetic data, when compared with geological evidence, can be most easily interpreted in terms of continental drift.

#### AGES OF ANCIENT MINERALS

G. R. TILTON, G. W. WETHERILL, L. T. ALDRICH,  
G. L. DAVIS,<sup>1</sup> AND P. M. JEFFERY<sup>2</sup>

In the report of this group a year ago three consistencies in the pattern of mineral ages obtained by the potassium-argon and rubidium-strontium methods had been found. First, the potassium-argon ages of micas of different grain size from the same rock unit were found to be in good agreement. Second, a similar agreement had been found for the rubidium-strontium ages of micas and potassium feldspars from the same rock unit. Finally, the ratio of the potassium-argon age to the rubidium-strontium age of micas from many different rock units had been found to be the same. These consistencies offered great promise that absolute potassium-

argon and rubidium-strontium ages could be found, especially since it was fairly obvious that the potassium-argon and rubidium-strontium ages could be made to agree simply by the use of decay constants for  $K^{40}$  and  $Rb^{87}$  that differ slightly from the values given in the usual tables of constants. This resolution of the problem was reasonable in view of the diverse values of these decay constants obtained by laboratory counting experiments; some justification had also been found from comparisons of concordant uranium-lead ages of uraninite and measurements of the parent-to-daughter ratios,  $A^{40}/K^{40}$  and  $Sr^{87}/Rb^{87}$ , of micas from the Bob Ingersoll pegmatite (South Dakota). This year we have extended the work begun on the Ingersoll pegmatite to four additional pegmatites, and from these data have determined values of the decay constants of  $K^{40}$  and  $Rb^{87}$ . The values found for these decay constants are within the range of values found by direct counting.

In the measurement of uranium-lead ages last year it was shown that diversity in the ages obtained on monazite, microcline, and zircon was to be expected. Thus any hope for unambiguous comparisons of ages by uranium-lead, potassium-argon, and rubidium-strontium analyses lay with pegmatites containing uraninite.

Analysis of material leached from monazite and microcline by cold acid indicated that the uranium-lead age pattern found could be explained by a similar, but probably slower, natural loss of lead and uranium. Both aspects of this work have been extended this year.

A summary of the progress this year is contained in the following statements:

1. It was learned that the age of a mineral less than 500 million years is very strongly dependent on  $\lambda_e$ , the decay constant for the decay of  $K^{40} \rightarrow A^{40}$ , and relatively independent of  $\lambda_\beta$ , the decay constant for  $K^{40} \rightarrow Ca^{40}$ . Using the young pegmatites in this study and measurements of Wasserburg and Hayden, one calculates that  $\lambda_e$  should be  $0.557 \times 10^{-10} \text{ yr}^{-1}$ . Tak-

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<sup>2</sup>Has returned to University of Western Australia, Nedlands, Western Australia.



ing this value for  $\lambda_e$ , and an average of the best counting experiments for  $\lambda_\beta$ , it is found that the K-A ages of micas agree very well with the concordant U-Pb ages of the uranium minerals from the same pegmatite, as is shown in figure 10.

2. The decay of  $\text{Rb}^{87}$  could also be studied on the same minerals as the decay of  $\text{K}^{40}$  and, in addition, feldspars could be

very thin films of rubidium chloride have given results for the half-life of  $\text{Rb}^{87}$  that agree exactly with the value found by the geological method we have used.

3. From items 2 and 3 it is seen that the K-A and Rb-Sr ages of micas are consistent over the whole range of geologic time and, with proper choice of decay constants, with each other and with the

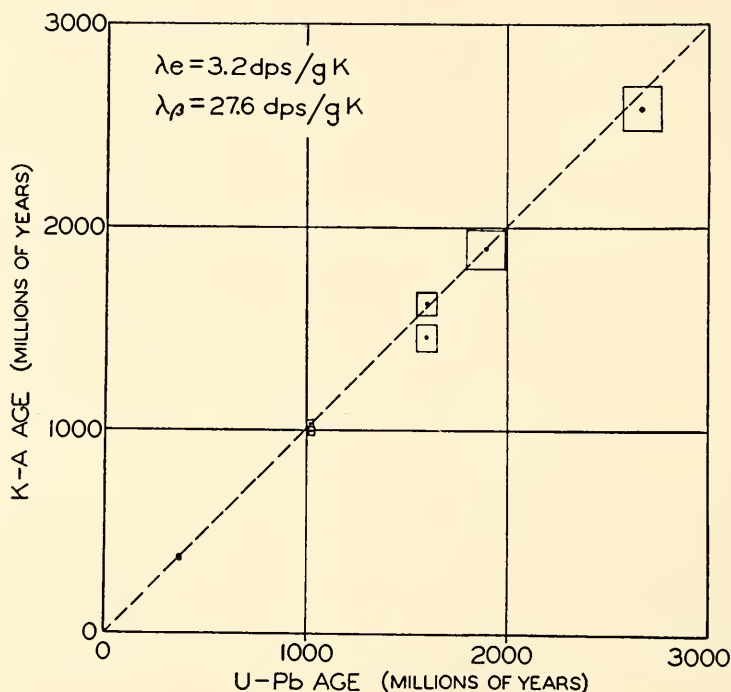


FIG. 10. Potassium-argon ages of micas vs. concordant uranium-lead ages of six pegmatites.

used to determine the ratio radiogenic  $\text{Sr}^{87}/\text{Rb}^{87}$  characteristic of the pegmatite. These ratios are plotted on figure 11 against the concordant U-Pb age of the pegmatite. The dotted line through the points is the position one would expect for the points if the half-life of  $\text{Rb}^{87}$  were 50 billion years. The two solid lines show where the points should lie for extreme values of this half-life as determined by counting experiments. We have recently heard from E. Huster, of the Physikalisches Institut of the University of Marburg, that his counting experiments using

U-Pb ages of the rock unit. This comparison has been extended to some 24 different granites and pegmatites for which ratios  $\text{A}^{40}/\text{K}^{40}$  and  $\text{Sr}^{87}/\text{Rb}^{87}$  had previously been obtained but for which U-Pb ages either were not available or were discordant, as in the case of the Brown Derby pegmatite discussed last year. Using the decay constants found above with these ratios, K-A and Rb-Sr ages of micas were obtained for these 24 rock units; figure 12 is a histogram of their ratio. It is seen first that five-sixths of the rock units display ages that agree within 10 per cent. Sec-



ond, the cases in which the agreement is poorer could be explained by argon leakage from the mica.

4. A straightforward method of analysis has been devised whereby discordant U-Pb ages of different uranium minerals from the same formation may be used to learn the possible history of the formation.

5. Further extension of the investigation of zircon as an age indicator of granite has demonstrated that this mineral often

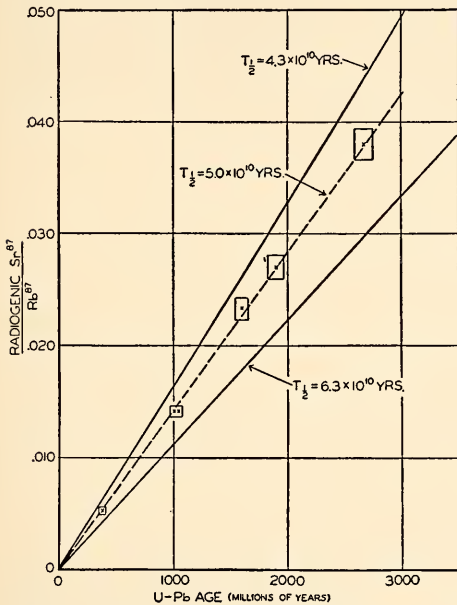


FIG. 11. Ratio radiogenic  $\text{Sr}^{87}/\text{Rb}^{87}$  vs. concordant U-Pb ages of six pegmatites.

does not fulfill the requirements for reliable age determinations.

6. An investigation of the ratio  $\text{Pb}/\text{U}$  and the isotopic composition of lead found in ultramafic nodules has shown that they are not undifferentiated samples of the earth's mantle. These rocks sometimes have a lead isotopic composition distinctly different from that of the basalt which surrounds them—an indication that the nodules and the basalt have sources with different ratios of lead to uranium for some recent part of their history.

The results summarized in items 1-3

above indicate that we are now in a very strong position to determine the ages of igneous intrusives using K-A and Rb-Sr ages of micas. The first phase of our studies is thus completed. For those cases for which the Rb-Sr and K-A ages agree, all previous measurements can now be given in terms of an absolute age. It is our present intent to apply the results of this work to the geological problems amenable to this approach. Our future collections will be made with this emphasis rather than with that of obtaining minerals through which different methods of mineral age determinations may be compared.

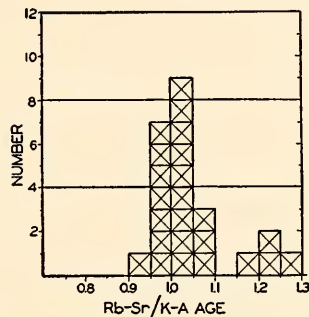


FIG. 12. Histogram showing ratio of K-A age to Rb-Sr age of micas from 24 different granites and pegmatites.

The data to support the statements above are for the most part in various stages of publication. They will be briefly discussed in the following paragraphs.

#### *Concordant Uranium-Lead Ages of Uraninite*

Four new uraninite analyses have been added to that from the Bob Ingersoll Mine presented last year. The analyses gave the U-Pb and Th-Pb ages shown in table 2. The reason for an occasional low Th-Pb age is as yet unknown, but it is our experience that almost all thorium ages have been low. The two U-Pb ages on four of the uraninites agree within 2 per cent, and on the fifth they agree within 3 per cent. These five analyses provide us with five pegmatites whose ages are known quite

precisely, so that K-A and Rb-Sr measurements may be used with these ages to determine the constants governing the decay of  $K^{40}$  and  $Rb^{87}$ .

### *Potassium-Argon Ages of Pegmatites and Decay Constants of $K^{40}$*

Previous annual reports have described the results of comparisons between K-A ages and Rb-Sr and U-Pb ages of the same rock. It was found that there was a strong correlation between K-A ages and Rb-Sr ages for mica, and that the K-A ages could be fitted to the few concordant U-Pb data with a reasonable choice of  $K^{40}$  decay constants. These facts made it seem promising that K-A measurements would give

The measured age is equally sensitive to the choice of these two decay constants for an age of 3500 million years. Thus, for the purpose of mineral age determination, it is primarily necessary to know  $\lambda_e$ , and accurate knowledge of  $\lambda_\beta$  is less important. Previous discussion of this problem has centered on the branching ratio  $\lambda_e/\lambda_\beta$ . To neglect the relative importance of the two decay constants as discussed above can be confusing. In fact, an increase in the branching ratio may cause either a decrease or an increase in the calculated age, depending on whether the increase in the branching ratio is caused by an increase in  $\lambda_e$  or a decrease in  $\lambda_\beta$ .

Since the dependence of the measured

TABLE 2  
URANIUM-LEAD AND THORIUM-LEAD AGES OF URANINITES FROM FIVE PEGMATITES

LOCATION	AGE (MILLION YEARS)			
	U <sup>238</sup> -Pb <sup>206</sup>	U <sup>235</sup> -Pb <sup>207</sup>	Pb <sup>207</sup> -Pb <sup>206</sup>	Th <sup>232</sup> -Pb <sup>208</sup>
Chestnut Flat Mine, Spruce Pine, N. C. ....	370 ± 10	375 ± 10	.....	.....
Fission Mine, Wilberforce, Ontario. ....	1040 ± 30	1050 ± 30	1070 ± 50	1010 ± 30
Cardiff Mine, Cardiff Township, Ontario. ....	1020 ± 30	1020 ± 30	1020 ± 30	995 ± 30
Bob Ingersoll Mine, Keystone, S. D. ....	1580 ± 30	1600 ± 30	1630 ± 30	1440 ± 50
Viking Lake Pegmatite, Saskatchewan. ....	1790 ± 50	1830 ± 50	1870 ± 50	1640 ± 50

absolute ages for mica if the correct decay constants could be found.

$K^{40}$  has two decay constants:  $\lambda_e$  represents the probability of decay by electron capture to form  $A^{40}$ ;  $\lambda_\beta$  represents the probability of decay by beta-particle emission to form  $Ca^{40}$ . The K-A age of a mineral is related to these decay constants by the equation:

$$\tau = \frac{1}{\lambda_e + \lambda_\beta} \ln \left( \frac{A^{40}}{K^{40}} \frac{\lambda_\beta + \lambda_e}{\lambda_e} + 1 \right)$$

where  $\tau$  is the calculated K-A age, and  $A^{40}/K^{40}$  is the ratio of the concentrations of these two isotopes in the mineral. From this equation it can be shown that for young minerals  $\tau$  is almost entirely dependent on  $\lambda_e$  and that even for minerals 1300 million years old the dependence of  $\tau$  on variations in the choice of  $\lambda_e$  is three times its dependence on variations of  $\lambda_\beta$ .

age on the experimental uncertainty in  $\lambda_\beta$  is negligible for young minerals, comparison of concordant uranium ages with measured  $A^{40}/K^{40}$  ratios of mica from the same rock permits the calculation of  $\lambda_e$ . The result of this calculation, using the data obtained in this laboratory together with those of Wasserburg and Hayden, is the value  $\lambda_e = 0.557 \times 10^{-10} \text{ yr}^{-1}$ , corresponding to a specific gamma activity of 3.2  $\gamma/\text{gr}/\text{sec}$  for natural potassium. Then, by choosing a reasonable value for the specific beta activity,  $\lambda_\beta = 27.6 \beta/\text{gr}/\text{sec}$  (the average given by Endt and Kluyver), it is found that good agreement obtains between K-A ages of mica and concordant U-Pb ages over a great span of time, as is evident in figure 10. These data are also shown in table 3.

This value of the specific gamma activity is not in disagreement with the ex-

perimental values, which range from 2.6 to 3.6  $\gamma/\text{gr}/\text{sec}$ . Perhaps the best experimental determination in the literature is that of McNair, Glover, and Wilson, who obtain a result of 3.33  $\gamma/\text{gr}/\text{sec}$  using  $\text{Na}^{24}$  to calibrate a  $\text{NaI}(\text{Tl})$  scintillation spectrometer. A similar experiment is being conducted here, using not only  $\text{Na}^{24}$  but also the two gamma rays of  $\text{Co}^{60}$  as standards. Preliminary results are in agreement with those of McNair, Glover, and Wilson, and it seems that the actual specific gamma activity may be slightly higher than 3.2  $\gamma/\text{gr}/\text{sec}$  and that a few per cent of the radiogenic argon have been lost by the mica. The discrepancy, however, is just

the method employed by our group. From 1 kg of lepidolite whose U-Pb age was thought to be  $2 \times 10^9$  years, strontium was extracted and weighed, as was the rubidium. Approximately 156 mg of  $\text{Sr}^{87}$  were recovered from the 25.7 gr of rubidium in the mineral. From the age, the isotopic abundance of  $\text{Rb}^{87}$  available, and these two weighings, the decay constant is calculated to be  $6.3 \times 10^{10}$  years. Subsequent measurements on the same lepidolite indicate that the Sr obtained was only about 60 per cent of that actually in the mineral. Further measurements of the U-Pb ages indicate that the true age of the pegmatite is  $2.5 \times 10^9$  years, and that

TABLE 3

POTASSIUM-ARGON, RUBIDIUM-STRONTIUM, AND CONCORDANT URANIUM-LEAD AGES OF SIX PEGMATITES

LOCATION	AGE (MILLION YEARS)		
	K-A	Rb-Sr	U-Pb
Chestnut Flat Mine, N. C. ....	$350 \pm 15$	$375 \pm 20$	$375 \pm 10$
Fission Mine, Ontario. ....	$960 \pm 50$	$1000 \pm 50$	$1050 \pm 30$
Cardiff Mine, Ontario. ....	$1000 \pm 50$	$1030 \pm 50$	$1020 \pm 30$
Ingersoll Mine, S. D. ....	$1600 \pm 60$	$1650 \pm 60$	$1600 \pm 30$
Viking Lake, Saskatchewan. ....	$1850 \pm 80$	$1970 \pm 50$	$1830 \pm 50$
Bikita Quarry, S. Rhodesia. ....	$2550 \pm 150$	$2680 \pm 100$	2680 *

\* Holmes, *Nature*, vol. 173, p. 612 (1954).

at the limit of experimental error of the two methods and may not be real.

In any case, the K-A ages will not be far from being absolute ages, although they may be a few per cent low when the correct decay constants are used. If larger amounts of argon are lost, the fact will almost certainly show up as a discrepancy between the Rb-Sr and K-A ages, and can therefore be recognized. As has been pointed out in the introduction, these discrepancies are relatively rare, and the agreement of these two ages is a useful criterion for acceptance of the absolute age of a sample of mica.

### *The Half-Life of $\text{Rb}^{87}$*

One of the first modern measurements of this physical constant was made by Hahn and his co-workers, using essentially

these two errors partly cancel to give a value close to the true decay constant of  $\text{Rb}^{87}$ .

Almost all subsequent counting experiments have agreed with the value found by Hahn and his co-workers. Two such experiments gave values which were 4.0 and  $4.3 \times 10^{10}$  years, about 40 per cent lower than the others. The value  $4.3 \times 10^{10}$  years was obtained by the careful work of E. Huster and his co-workers at the University of Marburg by extrapolation of thin-source counting data. We have very recent word from Huster that counting still thinner films has shown his original extrapolation to be in error, and he now considers 4.9 to  $5.0 \times 10^{10}$  years to be the best value for this constant. Figure 11 shows that our data on  $\text{Sr}^{87}/\text{Rb}^{87}$  are fitted best by a value  $5.0 \times 10^{10}$  years. Counting

data and geological measurements are thus reconcilable in a consistent and most satisfactory way. We have, then, found the decay constants which make Rb-Sr ages absolute ages whenever the other requirements for valid mineral ages are fulfilled.

From the agreement of the K-A ages with U-Pb ages, and Rb-Sr ages with these same U-Pb ages, there follows at once the agreement of K-A ages with Rb-Sr ages of micas from this group of pegmatites, as is seen in table 3. These six pegmatites can now be safely considered to have the

12. They are presented in more detail in the following tables. They are grouped according to the information available for each granite or pegmatite, so that comparisons may be made more readily. Data for the six pegmatites that served as a basis for this study have already been presented.

Table 4 gives K-A, Rb-Sr, U-Pb, and Th-Pb ages of two pegmatites for which the U-Pb ages are not concordant. Table 5 gives K-A, Rb-Sr,  $U^{238}$ - $Pb^{206}$ ,  $Pb^{207}$ - $Pb^{206}$ , and  $Th^{232}$ - $Pb^{208}$  ages of various minerals from granites for which these ages are available. The U-Pb ages of the zircons

TABLE 4  
COMPARATIVE AGES OF TWO PEGMATITES

Location	Mineral	K-A	Rb-Sr	$U^{238}$ - $Pb^{206}$	$U^{235}$ - $Pb^{207}$	$Pb^{207}$ - $Pb^{206}$	$Th^{232}$ - $Pb^{208}$
Brown Derby Mine, Gunnison Co., Colo.	Mica (avg. of 5)	1360	1410	.....	.....	.....	.....
	Microcline	830	1290	.....	.....	.....	.....
	Microlite	.....	.....	915	1055	1350	.....
	Monazite	.....	.....	1590	1420	1170	995
	Columbite- tantalite	.....	.....	1520	1470	1390	.....
Harding Mine, Dixon, N. Mex. ....	Mica (avg. of 2)	1300	1300	.....	.....	.....	.....
	Microlite (light)	.....	.....	970	960	940	.....
	Microlite (dark)	.....	.....	920	1000	1130	.....

most reliable ages yet measured for any rock units.

#### *Potassium-Argon and Rubidium-Strontium Ages of Granites and Pegmatites*

The decay constants for  $K^{40}$  and  $Rb^{87}$  discussed in the preceding sections have now been applied to measurements completed over the past several years. Many of these measurements were made on micas from rocks whose uranium minerals did not have concordant U-Pb ages so that they could not be used to find the decay constants of  $K^{40}$  and  $Rb^{87}$ . The previous measurements and those made this year are summarized in the histogram of figure

from these granites are discussed more fully later, but they are presented here for comparison. In general, the K-A and Rb-Sr ages agree well even when the U-Pb ages from the zircons disagree by quite large amounts. Because of the great chemical difference of the daughter elements in the two systems, the agreement of these two ages is believed to be a very strong criterion that the absolute age of the rock unit has been obtained. Table 6 gives K-A and Rb-Sr ages of granites and pegmatites for which no U-Pb ages have been measured.

A group of Australian samples for which K-A and Rb-Sr ages have been measured



TABLE 5  
AGES OF GRANITES

Location	Mineral	K-A	Rb-Sr	U <sup>238</sup> -Pb <sup>206</sup>	Pb <sup>207</sup> -Pb <sup>206</sup>	Th <sup>232</sup> -Pb <sup>206</sup>
Conway, N. H.....	Biotite	144	185	....	....	....
	Zircon	....	....	193	....	210
Cape Town, S. Africa.....	Biotite	530	595	....	....	....
	Zircon	....	....	330	530	238
Pikes Peak, near Manitou Springs, Colo. ....	Biotite	1030	1020	....	....	....
	Zircon	....	....	624	980	313
Uncompahgre, Mesa Co., Colo...	Biotite- xenotime	1360	1370	3180	1640	1100
Lawler Peak, Bagdad, Ariz.....	Muscovite	1410	1390	....	....	....
	Lepidolite	1410	1500	....	....	....
	Zircon	....	....	630	1210	270
Quartz Creek, Gunnison Co., Colo. ....	Biotite	1320	1310	....	....	530
	Zircon (A)	....	....	925	1540	....
	Zircon (B)	....	....	....	1700	....
Petrick Quarry, Llano Co., Texas ....	Biotite	1090	1100	....	....	....
	Zircon	....	....	950	1070	890

is particularly interesting. The ages measured are given in table 7. The greater part of Western Australia is supposedly of pre-Cambrian age, but few radioactive age determinations have been made to substantiate this conjecture. Samples from four pegmatite dikes were dated by the K-A and Rb-Sr methods and were found to be very early pre-Cambrian. Three of the dikes were separated geographically by about 800 miles, but their ages appear to be the same within the limits of experimental error. It is interesting to note that all these dikes, with the exception of that at Wodgina, intrude what is assumed to be the oldest rock system in Australia, the

"older greenstone series." From the data presented here the lower limit for the age of this series must be close to 2800 million years.

Three samples from tables 5 and 6 demonstrate the applicability of mineral age measurements to local geological problems: the granites from the Front Range of Colorado and southeast Wyoming, the two Pikes Peak granites, and the Sherman granite. The K-A and Rb-Sr ages of these granites are given again for convenience in table 8.

Field observations tentatively separated the two Pikes Peak granites, placing that from the top of Pikes Peak in the Creta-

TABLE 6  
POTASSIUM-ARGON AND RUBIDIUM-STRONTIUM AGES OF GRANITES AND PEGMATITES

Location	Mineral	K-A	Rb-Sr
Pegmatite, Jakkalswater, Namaqualand, Africa..	Lepidolite	970	960
Granite, from top of Pikes Peak, Colo.....	Biotite	1100	1080
Pegmatite, Wickenburg, Ariz.....	Lepidolite	1160	1300
Granite, SE. of Albuquerque, N. Mex.....	Biotite	1340	1300
Granite, NW. of Sherman, Wyo.....	Biotite	1410	1420
Pegmatite, N. of Bonneville, Wyo.....	Lepidolite	2550	2680

TABLE 7  
AGES OF MINERALS FROM WESTERN AUSTRALIA

LOCALITY	MINERAL	AGE (MILLION YEARS)	
		K-A	Rb-Sr
Catlin Creek . . .	Muscovite	2780	2810
	Microcline	2130	2700
Londonderry . .	Muscovite	2660	3190
	Microcline	2160	2760
Wodgina . . . . .	Muscovite	2370	2940
	Microcline	2160	1850
Grosmont . . . .	Lepidolite	2740	2640
	Microcline	2700	2740
	Lepidolite (inclusion in microcline)	2730	2800

ceous ( $\sim 100$  million years) and that near Manitou Springs at 1000 million years. The other field relationship which had been suggested was that the Sherman granite was intruded at the same time as that near Manitou Springs. From table 8 it can be said with some certainty that the two Pikes Peak granites are nearly the same age and that they in turn intruded the pre-Cambrian sediments of the area some 300 million years later than the Sherman granite.

Tables 3, 6, and 7 conclusively demonstrate that pre-Cambrian minerals of an age of 2500 to 2700 million years exist on three continents. Thus the total period of earth history is greater than this. This reckoning still leaves a gap of about 1500 million years between the ages of meteorites and that of the oldest terrestrial minerals. It would, of course, be interesting to

TABLE 8  
POTASSIUM-ARGON AND RUBIDIUM-STRONTIUM AGES  
OF THREE FRONT RANGE GRANITES

GRANITE	AGE (MILLION YEARS)	
	K-A	Rb-Sr
Pikes Peak (top) . . . . .	1100	1080
Pikes Peak (Manitou Springs) . . .	1030	1020
Sherman granite . . . . .	1410	1420

investigate this interval as completely as possible with terrestrial materials.

### *Interpretation of Discordant Uranium-Lead Ages*

One of the principal difficulties in establishing a uranium-lead time scale has been the fact that as a general rule the two independent uranium-lead ages do not agree with each other. Although samples of uraninite from pegmatites usually give concordant ages, most other uranium-bearing minerals, such as zircon, monazite, and microcline, seldom do.

The possible causes of this discordance are (1) losses or gains of uranium or lead elements during geological time, (2) losses of intermediate daughter products, such as radon, (3) the presence of primary radiogenic lead, and (4) analytic errors.

As a result of the development of modern isotope dilution technique, item 4 can be eliminated as an important source of discordance. Items 2 and 3 may be important occasionally, but comparison of discordant uranium-lead ages with absolute ages of the same rock determined by both the Rb-Sr and K-A methods indicates that these cannot be the sole causes of discordance. Present indications are that item 1 is the principal general cause of discordant uranium-lead ages, and this discussion will be primarily concerned with it.

The finding of discordant ages certainly makes the problem of uranium-lead dating more complex. The fact that the calculated ages have been affected by the history of the mineral, however, raises the possibility that the discordant data may be used to learn something of this history. The data may be handled analytically, but the mathematical expressions resulting from generalizations of the age equations to include the causes of discordance are very cumbersome. The interpretation of the discordant data is greatly facilitated by the following graphical procedure.

Figure 13 is a graph on which the ratio  $Pb^{206}/U^{238}$  is plotted on the ordinate and the ratio  $Pb^{207}/U^{235}$  on the abscissa. The

usual chemical and isotopic analyses of lead and uranium in a mineral suffice to determine a point on this diagram. All points representing concordant ages will be on the curve marked "concordia." For each concordant age there is a unique point, and a time scale may be laid out along the curve "concordia" as shown in figure 13. This type of diagram has a number of useful properties, of which two will be discussed.

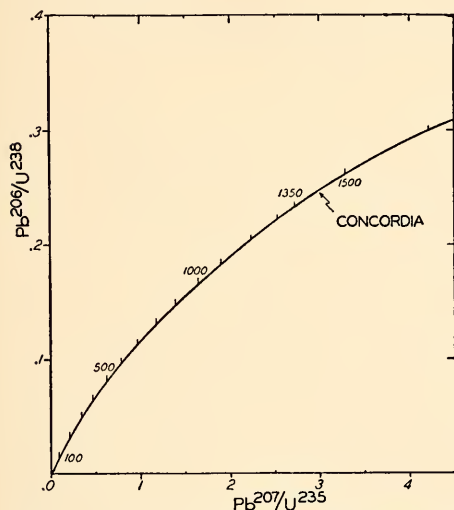


FIG. 13. Plot of  $Pb^{206}/U^{238}$  vs.  $Pb^{207}/U^{235}$ , which is useful for the interpretation of discordant U-Pb ages.

Suppose that a mineral were formed at some time in the past, for example 1000 million years ago. If no subsequent alterations took place, the point of figure 13 which is characteristic of this mineral would lie on the curve "concordia" at the 1000-million-year point. Now let us suppose that at some time subsequent to the formation of this mineral, for example 100 million years ago, half of the lead were lost from the mineral. The resulting point can be found by drawing a straight line between the point "1000" on concordia and the point "100" on concordia, and then taking the midpoint of this line. If, on the other hand, only one-third of the lead

were lost, the resulting point would lie on this same line, but now only one-third of the distance between the points "1000" and "100." For the case of loss of uranium at this time the resulting point will lie on the continuation of this same straight line above concordia. Thus, if a collection of minerals suffered varying degrees of alteration at the same time, the points obtained as a result of the analysis of these minerals will all lie on the same straight line; the upper intercept of this line with concordia will give the time of original mineralization, and the lower intercept will give the time at which the alteration took place.

This procedure provides a simple possible interpretation of the regularity discovered by Ahrens, shown in figure 14. The four points represent samples of monazite from Rhodesia and Madagascar. It was found by Ahrens that when plotted in this way the points all lay on a straight line. In accordance with the preceding discussion, these points could be interpreted as representing minerals which were formed 2700 million years ago and suffered some sort of alteration 500 million years ago. This graphical procedure can be readily generalized to include multiple episodes of uranium-lead fractionation, and all the results can be rigorously demonstrated.

The second application of this diagram that will be discussed is a method of finding the probable true age of a collection of minerals giving discordant ages even when no regularities exist. Figure 15 shows the area of the diagram accessible to points representing minerals having a true age of 1350 million years which have lost up to 50 per cent of their lead and up to 25 per cent of their uranium during geological time. It should be noted that this region forms a rather long, thin band pinching in toward the point on concordia representing the true age of the mineral. For other cases, for example up to 75 per cent lead loss, similar regions are defined. Natural minerals can be expected to have

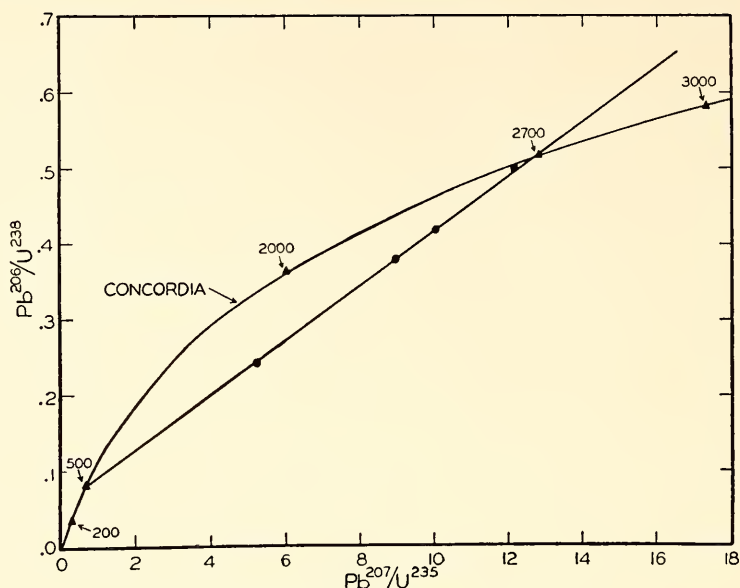


FIG. 14. The "Rhodesia age pattern" of Ahrens

experienced histories like this, and points representing these minerals may be expected to fall within bands of this kind. If a small number of minerals, say four, from the same rock are analyzed, and their characteristic points are plotted on this

points will intercept concordia at a point near the true age of the minerals.

The possible usefulness of this procedure is illustrated by a numerical example. We have considered a hypothetical collection of 48 mineral samples having a true age of 1350 million years and have randomly selected a hypothetical history for each mineral. The assumption was made that uranium-lead fractionation was equally probable at all times, and an arbitrary weighting was given to the probability of different degrees of uranium-lead fractionation, corresponding roughly to what seems reasonable from measurements of discordant ages of actual minerals.

The discordant  $U^{238}$ - $Pb^{206}$  ages are shown in figure 16(a), and are seen to scatter widely as a result of the assumed histories. Figure 16(b) shows what is found if only apparently concordant ages are used, with the common criterion for concordance of agreement between the  $U^{238}$ - $Pb^{206}$  and  $U^{235}$ - $Pb^{207}$  ages within 2 per cent. Although the scatter is reduced considerably, it is still large, indicating that accidental concordance is a real problem. This accidental concordance arises

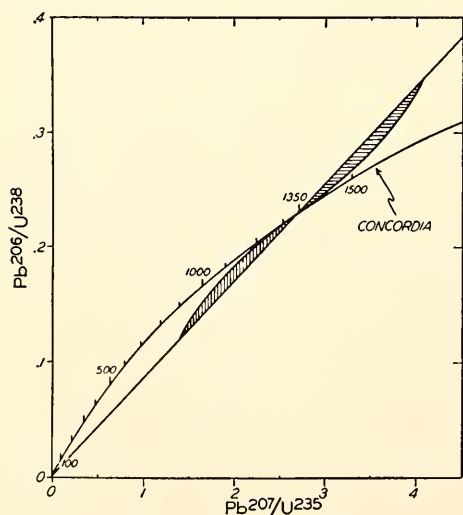


FIG. 15. Diagram showing the areas representing minerals that have lost up to 50 per cent of their lead or up to 25 per cent of their uranium. diagram, a straight line fitted to these



from the fact that a straight chord between two points on concordia will always lie close to concordia unless the two points are widely separated in time. Figure 16(c) shows the results obtained if the  $\text{Pb}^{207}\text{-Pb}^{206}$  ages are used, and illustrates

the relative probabilities of different degrees of uranium-lead fractionation.

Application of this method to the data obtained from minerals from the Brown Derby pegmatite (table 4) gives an age of 1340 million years. This age is in agree-

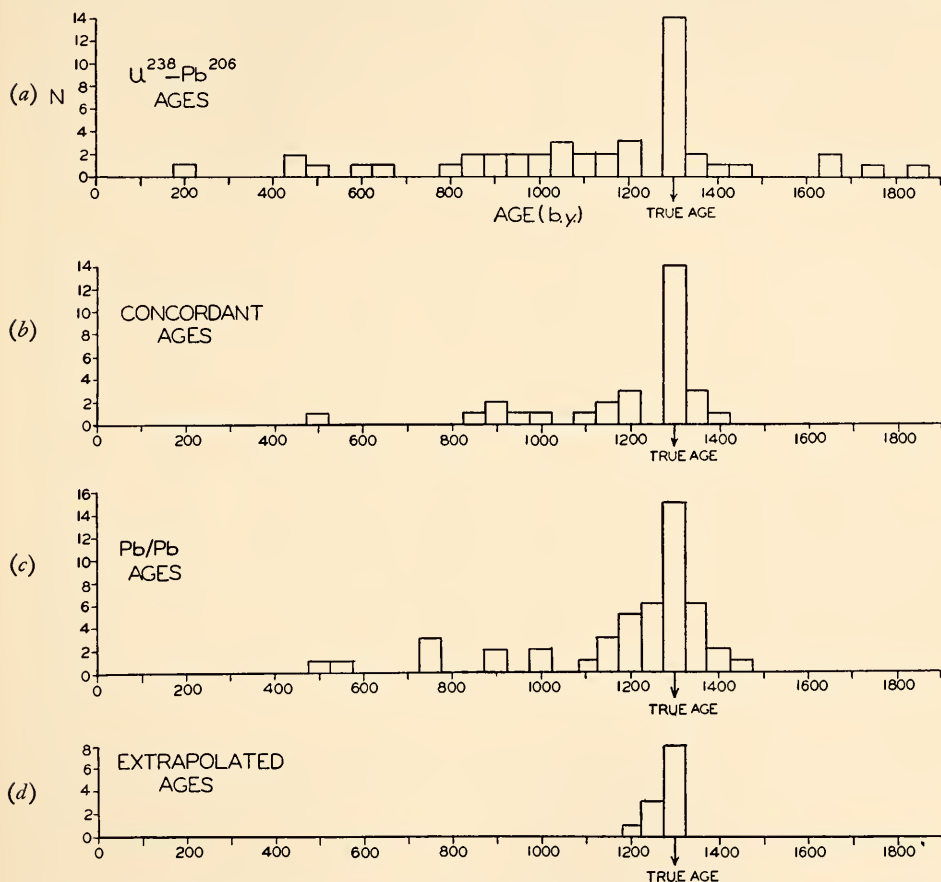


FIG. 16. (a)  $\text{U}^{238}\text{-Pb}^{206}$  ages resulting from the hypothetical losses of lead and uranium. (b) Accidental and true concordant U-Pb ages resulting from these histories. (c)  $\text{Pb}^{207}\text{-Pb}^{206}$  ages resulting from these histories. (d) "Extrapolated" ages obtained by method described in the text. The 48 histories were used in groups of four, allowing the calculation of twelve ages.

the well known fact that this age is less sensitive to uranium-lead fractionation than the others. In figure 16(d) are shown the ages obtained by the graphical procedure described above, the 48 separate histories being taken in groups of four each. The twelve ages found in this way cluster about the true age. Similar results will be found for other assumptions about

ment with the Rb-Sr and K-A ages given in table 4.

### Zircon Ages

Isotopic age work on zircons was extended in the past year to eleven samples. The work now constitutes a survey of the zircon age problem, from which some generalizations appear to emerge. The new

determinations in table 9 are the Pikes Peak, Petrick Quarry, Conway, and Cardiff Township specimens. The remaining results have appeared in previous reports.

The zircon ages in table 9 exhibit correlations with the localities in which they are found. The three zircons from the Grenville subprovince in Ontario have uranium-lead ages which are in reasonable agreement at 1000 million years. The material includes a range of crystal sizes from -200 mesh for the Tory Hill syenite to

pothesis accords with the observations that the Grenville in Ontario has been relatively undisturbed since the pre-Cambrian whereas the three granites from the western United States came from areas in which orogenies have occurred in the post-Cambrian. A generalization of this hypothesis is that the concordance or discordance of ages obtained from a zircon is a function of the post-crystallization history of the rock in which it is found rather than some intrinsic property of the crystals

TABLE 9  
LEAD CONTENT AND AGES OF ZIRCONS

LOCALITY	PRIMARY LEAD (PPM)	TOTAL LEAD (PPM)	AGE (MILLION YEARS)			
			U <sup>238</sup> .. Pb <sup>206</sup>	U <sup>235</sup> .. Pb <sup>207</sup>	Pb <sup>207</sup> .. Pb <sup>206</sup>	Th <sup>232</sup> .. Pb <sup>208</sup>
Tory Hill, Ontario (granite).....	≤5.5	461	1030	1050	1090	390
Tory Hill, Ontario (syenite).....	≤1.5	123	940	960	1015	...
Cardiff Twp., Ontario, Cardiff Uranium Mine .....	≤6	485	900	930	1000	990
Natural Bridge, N. Y., Ashmore Farm..	≤1.6	127	1025	1065	1140	...
Gunnison Co., Colo., Quartz Creek Dist..	54	210	925	1130	1540	530
Bagdad, Ariz. ....	40	338	630	770	1210	271
Pikes Peak, Colo., Manitou Springs.....	3	33.3	624	707	980	313
Llano Co., Texas, Petrick Quarry.....	≤0.6	53.8	950	990	1070	890
Conway, N. H.* .....	...	...	193	...	...	210
Cape Town, S. Africa.....	14	66.5	330	356	530	238
Ceylon (gem) .....	≤1.7	127	540	544	555	538

\* Preliminary result.

several millimeters for the Cardiff Township sample. As stated elsewhere in this report, two uraninites from this area also give concordant uranium-lead ages of about 1000 million years. On the other hand, three pre-Cambrian zircons from the Cordilleran region of the western United States (Bagdad, Quartz Creek, and Pikes Peak samples) all give discordant uranium-lead ages. Although future work with other zircons from the Cordilleran System may very well reveal some concordant uranium-lead ages, there appears to be considerable truth to the generalization that the Grenville subprovince is a more favorable locality in which to measure ages by zircons than is the Cordilleran region of western United States. This hy-

pothesis accords with the observations that the Grenville in Ontario has been relatively undisturbed since the pre-Cambrian whereas the three granites from the western United States came from areas in which orogenies have occurred in the post-Cambrian.

Another interesting difference exists between zircons having concordant and discordant ages. The seven zircons in table 9 which have Pb<sup>207</sup>-Pb<sup>206</sup> ages differing by less than approximately 10 per cent from their respective U<sup>238</sup>-Pb<sup>206</sup> ages also have no detectable primary lead. The actual analyses showed an amount of primary lead that was within the amount to be expected from the lead contamination obtained in chemical processing of the samples (about 0.5 microgram). The remaining four zircons which have Pb<sup>207</sup>-Pb<sup>206</sup> and U<sup>238</sup>-Pb<sup>206</sup> ages differing by about a factor of 2 had amounts of primary lead that definitely exceeded the

quantities that could be accounted for by the blanks. There is, then, an indication that the "trade mark" of the process causing zircon ages to be discordant may be the addition of some primary lead in the samples. Experiments have shown that the primary lead is so strongly bound that it cannot be removed by prolonged boiling of the crystals in concentrated nitric acid. It therefore appears necessary to keep open the possibility that the discordant ages found in zircons may result from addition of uranium and thorium with some primary lead to the samples rather than from loss of lead. Future progress in this study will evidently require close petrographic examination of samples in order to determine whether the primary lead resides in the crystal structure of the mineral or in inclusions and imperfections in the crystals.

The pattern of the age discrepancies continues to be the same as found in previous work, namely,  $Pb^{207}\text{-}Pb^{206} > U^{235}\text{-}Pb^{207} > U^{238}\text{-}Pb^{206} > Th^{232}\text{-}Pb^{208}$ . When such a pattern exists, K-A and Rb-Sr age determinations on associated micas indicate that the  $Pb^{207}\text{-}Pb^{206}$  age is the more nearly correct age for the zircons. The  $Pb^{207}\text{-}Pb^{206}$  age is less than the K-A and Rb-Sr ages at Bagdad and greater than the K-A and Rb-Sr ages at Quartz Creek, and for this reason it is unwise to give the  $Pb^{207}\text{-}Pb^{206}$  age a blanket endorsement as being always the "best" one for any zircon that may be studied in the future. It is unfortunate that no use has been found for the low  $Th^{232}\text{-}Pb^{208}$  ages other than to prescribe a possible upper limit on the time elapsed since the zircons were subjected to the processes that caused the discordant ages. Undoubtedly, detailed studies of the cause of zircon age discrepancies will make use of the thorium age information.

In summary, zircons give unambiguous ages in some instances but in others they yield age data requiring interpretation in order to fix an age. The eleven samples studied to date have established no criteria that will predict in advance which type of data will result, at least until several

analyses have been accumulated from a given locality. In general, the post-crystallization history of the particular assemblage in which the mineral resides seems to determine the age pattern. It will be interesting to observe whether discordant ages continue to correlate with the presence of tightly bound primary lead in the samples.

### *Geochemistry of Uranium and Lead*

Two years ago the isotopic composition of lead in two olivine nodules was reported. It was pointed out at that time that the uranium-to-lead ratios in these nodules is such that the isotopic composition of their lead would not have changed appreciably in the past four to five billions of years if they had always contained their present amounts of uranium and lead. Actually, the nodules contain a "modern" lead which must have evolved in an environment in which the uranium-to-lead ratio was some twenty times greater until recent times than that of these nodules today. Further studies now include the analyses of an additional nodule at Hualalai, Hawaii (by C. C. Patterson at the California Institute of Technology), a dunite (which is a massive assemblage of the same minerals that constitute the olivine nodules), and the basalt which enclosed the nodule at Hualalai. The results are summarized in table 10.

The leads from the three nodules and the dunite in table 10 are remarkably similar considering their diverse geographic origins. They are not identical, since their  $Pb^{206}/Pb^{207}$  ratios vary from 1.23 to 1.27 while the experimental accuracy of this ratio measurement is about  $\pm 0.5$  per cent. Nevertheless, the striking features of these leads are their similarities rather than their differences.

The fact that the nodules and the dunite have recently lost uranium with respect to lead suggests that they are formed by fractional crystallization. Since the isotopic composition of the lead in the basalt at

Hualalai has small, but significant, differences from that of the lead in the nodule, the basalt is eliminated as a possible parent for the nodule. Further studies will be made, particularly with basalts, in an effort to find materials from which the

nodules and the dunite could have been derived. The present studies have eliminated the possibility that ultramafic rocks are undifferentiated samples of the earth's mantle, but have not completely resolved the problem of the origin of these rocks.

TABLE 10

URANIUM, THORIUM, AND LEAD CONTENT, AND ISOTOPIC COMPOSITION OF LEAD FROM BASALTS AND ULTRAMAFIC ROCKS

SOURCE	SAMPLE	PARTS PER MILLION			ATOM RATIOS		
		U	Th	Pb	Pb <sup>206</sup> / Pb <sup>204</sup>	Pb <sup>207</sup> / Pb <sup>204</sup>	Pb <sup>208</sup> / Pb <sup>204</sup>
Dreiser Weiher, Germany, nodule	Diopside	...	...	2	19.43	15.30	37.86
San Bernardino, Calif., nodule	Diopside	0.007	...	2	19.16	15.46	37.78
Twin Sisters, Wash., dunite	Dunite minus spinel	0.016	0.05	1.6	19.17	15.61	38.15
Hualalai, Hawaii, nodule*	Nodule minus spinel	0.005	...	1	19.29	15.45	37.95
Hualalai, Hawaii, basalt	Basalt minus spinel	0.50	1.60	2.6	18.62	15.61	38.36

\* Analysis by C. C. Patterson, California Institute of Technology.

## THEORETICAL AND STATISTICAL GEOPHYSICS

### VARIABILITY OF LOWER IONOSPHERIC WIND SPEEDS INFERRED FROM GEOMAGNETIC EFFECTS OF SOLAR FLARES

S. E. FORBUSH

It has been known for several decades that the main features of the diurnal variation in the geomagnetic field arise from comparatively simple large-scale patterns of electric currents flowing in the ionosphere. According to the generally accepted dynamo theory, these currents are generated by horizontal winds which move the conducting air (in the lower ionospheric region at a height of 95 km or so) across the vertical component of the geomagnetic field.

An important contribution was made at the Department of Terrestrial Magnetism when McNish showed, in 1937, that the transient geomagnetic effects called crochets, which accompany more intense solar

flares or chromospheric eruptions, were indistinguishable from those that would result from a transient increase in strength of the current system responsible for the normal geomagnetic diurnal variation. This increase in strength of the current system is due to an increase in the conductivity produced by the flare radiation at or near the level of the diurnal-variation current system.

The amplitude of the solar geomagnetic diurnal variation,  $S_q$ , may vary as much as 100 per cent or so during a month; it also increases about 100 per cent with sunspot cycle. Bartels showed that no known geophysical variable is better correlated with sunspot numbers than the amplitude of the horizontal geomagnetic component of  $S_q$  at Huancayo. Nevertheless, this correlation is far from perfect for daily values of sunspot numbers and of the amplitude



of  $S_q$ . This variability in the amplitude of  $S_q$  may arise from variations in the component of solar radiation which determines the conductivity of the layer (below the E layer of the ionosphere) in which flow the currents responsible for  $S_q$ . On the other hand, day-to-day variations in the strength of the wind system, which according to the dynamo theory drives the  $S_q$  current-system, would also result in variations in the amplitude of  $S_q$ . Whether one or both of these effects is responsible for the variability of  $S_q$  has heretofore not been known.

During the 12-year period 1936-1947, 97 crochets were recorded at Huancayo in the horizontal geomagnetic component ( $H$ ) between the 0900<sup>h</sup> and 1400<sup>h</sup> 75° WMT. Between these hours the diurnal-variation departures, from night-time base values, are larger than for any other time of day; no crochets are observed at night. The 97 days with  $H$  crochets were dichotomized according to the size of the normal diurnal variation,  $S_q$ , in  $H$ . The average  $H$ -crochet size was greater by a statistically significant amount for the group of days with larger  $S_q$  amplitude in  $H$ . In addition, for the two groups of days the ratio of crochet sizes was about the same as the ratio (1.6) for the amplitudes of  $S_q$  in  $H$ . These findings indicate that on the average the strength of the wind system is greater on days with large  $S_q$ . Thus a part of the variability in the amplitude of  $S_q$  is due to variations in the strength of the dynamo wind system. The amplitude of  $S_q$  is known to increase with sunspot number. If the size of  $H$  crochets were also correlated with sunspot number, the correlation found between size of  $H$  crochet and amplitude of  $S_q$  might arise because both were influenced by causes related to sunspot numbers.

Statistical tests gave no correlation between the size of  $H$  crochets and sunspot number. The results of this study also indicate the means to provide a reliable relative scale for different solar flares, for the quantity of flare radiation in that range of the spectrum within which it is effective

in enhancing the conductivity of the  $S_q$  current system. It is planned to determine whether such "calibrated intensities" of solar-flare effects are correlated with absorption measurements in the lower ionosphere.

#### COSMIC-RAY INVESTIGATIONS

S. E. FORBUSH

*New solar-flare effect.* The fifth and largest increase in cosmic-ray intensity yet recorded at Cheltenham began at 03<sup>h</sup> 48<sup>m</sup> GMT on February 23, 1956, within 18 minutes after the beginning of a large solar flare or chromospheric eruption. By about 04<sup>h</sup> 05<sup>m</sup> the intensity had increased to a maximum at approximately 85 per cent above normal. A more detailed and complete record of the increase was obtained from the large ionization chamber at Derwood. The increase was also observed at Godhavn (Greenland), Christchurch (New Zealand), Ciudad Universitaria (Mexico), and Huancayo (Peru). This solar-flare increase was unusual in that it is the first known to contain charged particles, doubtless coming from the sun, of sufficient energy (at least 15 bev, if protons) to reach the earth at the geomagnetic equator. The maximum of the increase at Huancayo was about 18 per cent (average for 15 minutes), and that at Ciudad Universitaria was about twice as large.

As was pointed out in last year's annual report, theory indicates that, if the charged particles responsible for the increase come from the sun, no increase should be observed within about 30° of either geomagnetic pole. All five large solar-flare increases in cosmic-ray intensity thus far observed have involved increases at Godhavn, geomagnetic latitude 80°. The reasons for this are not known.

*Variations in cosmic-ray intensity with sunspot cycle.* The reduced data available from Huancayo and from Cheltenham both show a maximum in cosmic-ray intensity during the year 1954 when the sunspot number was a minimum. The previous maximum in yearly means of

cosmic-ray intensity occurred in 1944 during a minimum of sunspot numbers. Moreover, the Huancayo monthly indices of cosmic-ray activity, i.e. the standard deviation of daily means from the monthly means, were minimal in 1954. In all but two months of 1954, the index was scarcely larger than that which results from the "noise level" of the instrument. The fact that the yearly activity index for 1954 (from the pooled monthly indices) was unchanged when the five magnetically most disturbed days were omitted shows that the year was exceptionally free from day-to-day variability in cosmic-ray intensity. The previous yearly minimum in cosmic-ray activity coincided with the year of sunspot minimum of 1944.

*Twenty-seven-day variation in cosmic-ray intensity and in magnetic activity.* Cosmic-ray data from Huancayo, for a period of 250 solar rotations of 27 days each, have been harmonically analyzed (using a simple six-ordinate scheme) to obtain the phase and amplitude of the 27-day waves for successive rotations. For the same 250 solar rotations a similar analysis of international magnetic character figures was made. The phase of the waves in cosmic-ray intensity relative to that for character figure was then computed for each of the 250 rotations. The results show that the maxima of the cosmic-ray waves have a statistically significant tendency to occur near the times of the minima of the waves in magnetic character figure. The data were sorted into three groups according to the amplitude of the character-figure waves. For each of the three groups the maxima of the 27-day waves in cosmic-ray intensity tended with statistical significance to occur near the minima of the 27-day waves in character figure. In addition, the amplitude of the average cosmic-ray waves (with phases relative to the phases of the waves in character figure) from the three groups were correlated with the average of the character-figure amplitudes for the corresponding groups. Thus far, no evidence has been obtained to indicate that

maxima for 27-day waves in cosmic-ray intensity occur at times near the maxima in waves for magnetic activity, as has been reported by some investigators from analysis of insufficient data. Results are under study for years of high and low sunspot number.

*Old cosmic-ray program.* Compton-Bennett meters were satisfactorily operated throughout the report year at Godhavn (Greenland), Cheltenham (Maryland, U. S.), Climax (Colorado, U. S.), Ciudad Universitaria (Mexico, D. F.), Huancayo (Peru), and Christchurch (New Zealand). Tabulations of bihourly means of ionization corrected for bursts and barometric pressure for Huancayo are ready for publication from 1946 to 1955, and for Cheltenham from 1937 to 1954, as well as summaries for Godhavn and Christchurch. Publication of these results, in collaboration with the Instituto Geofísico de Huancayo, together with those contained in C. I. W. Publication 175, will make available to investigators most of the essential data obtained since the start of the Department's cosmic-ray program.

*Large ionization chamber.* The large cosmic-ray ionization chamber was maintained in essentially continuous operation at Derwood during the report year.

As mentioned in connection with the new solar-flare effect, an excellent record of this increase in cosmic-ray intensity was obtained with the Derwood ionization chamber, from which intensity averages over periods of a few minutes were reliably obtained. These data doubtless comprise one of the most complete and detailed ionization-chamber records of cosmic-ray intensity during this solar flare.

*Co-operation in operation of cosmic-ray meters.* The successful operation of Compton-Bennett cosmic-ray meters over a long period at so many stations has been possible only through the wholehearted and unselfish co-operation of several organizations and individuals. We wish to express our appreciation to the following organizations for the operation and maintenance

of cosmic-ray meters: The Danish Meteorological Institute and the staff of its Godhavn Magnetic Observatory at Godhavn, Greenland; the U. S. Coast and Geodetic Survey and the staff of its magnetic observatory at Cheltenham, Maryland; the High Altitude Observatory of the University of Colorado and its staff at Climax, Colorado; the Instituto Nacional de la Investigación Científica and the Universidad de Mexico, Mexico, D. F.; the Government of Peru and the staff of its Instituto Geofísico de Huancayo for making available the Compton-Bennett records from Huancayo; and the Department of Scientific and Industrial Research and the staff of its Magnetic Observatory at Christchurch, New Zealand.

#### EFFECT OF E-REGION WINDS UPON F-REGION HEIGHTS NEAR NOON

E. H. VESTINE

The semidiurnal tides near and within the E region of the atmosphere give rise to an air flow across the vertical component of the geomagnetic field. By dynamo action there results an electric field proportional to the product of the wind speed and the vertical geomagnetic field. Since the latter field is equal to zero at the magnetic equator, the current produced there would also be zero. The electric fields generated by the winds in middle latitudes, however, are such that they drive positive electric charges equatorward and westward in the morning, and electrons equatorward and eastward in the evening. This electric field, directed from west to east along the magnetic equator, also extends to the F region. Hirono, Maeda, and Martyn have suggested that this eastward electric field, crossed with the northward-directed geomagnetic field, must drive the F-region electrons and positive ions, alike, bodily upward. Hence, day-to-day changes in F-region height may afford a rough means of measuring the day-to-day variability of E-region wind speeds. Moreover, the use of day-to-day difference helps minimize

difficulties in interpreting complex hour-to-hour changes studied by Hirono.

In the same way the day-to-day differences in the noon penetration frequency  $f_oF_2$  for radio waves, which depend upon the height-sensitive electron concentration in the F region, can possibly be used to estimate wind-speed differences day by day, as well as the possible changes of these wind speeds with sunspot cycle.

If so, the day-to-day ranges in  $f_oF_2$  should on an average be simply related to day-to-day ranges in the solar daily magnetic variation  $S_q$ , provided that averages are taken such that effects of day-to-day changes in the electric conductivity of the E region are minimized.

On this basis it was found that the day-to-day differences in wind speed near and within the E region do not appear to change much with sunspot cycle, although the change in wind speed from one day to the next may be considerable. The change in  $f_oF_2$  with  $S_q$  is at the rate of about  $-0.2$  megacycle per gamma, whereas both  $f_oF_2$  and  $S_q$  increase with sunspot number, the corresponding increase in  $f_oF_2$  being about  $0.08$  megacycle for each gamma increase in  $S_q$ . The results found evidently confirm the dynamo theory, since effects on the F region proportional to wind speed in the E region, but independent of the conductivity there, give rise to proportional changes in  $S_q$ . They also accord with those found by Forbush, from results based on crochets referred to above.

#### EARTH'S CORE MOTIONS AND SHIFT OF THE GEOGRAPHIC POLES

E. H. VESTINE

In a previous study it was inferred that the gradual motion of the earth's eccentric dipole from south of the equator to north of the equator after about 1860 might imply a surface motion of the earth's central core about an axis in the equatorial plane. Another interpretation could be that harmonic terms of degree up to and



including two combine to yield a magnetic field pattern rotating in this fashion. From various dynamic checks based on the westward drift it now seems likely that the core can move relative to the mantle. It is hence of interest to discover, if possible, whether higher-degree harmonic terms drift transverse to the magnetic equator as do the terms of degree one and two.

Examination of irregularities in field pattern which could be identified sufficiently well showed that in the Atlantic area the drift of the eccentric dipole pattern seems verified since about 1785. In other words, in meridians where rapid motion of the eccentric dipole pattern occurs, a substantial motion of an Atlantic focus is found in declination, in the same sense, or to the south, as for the field pattern of the eccentric dipole since 1830. Munk has pointed out an error in the writer's previous estimates of gyroscopic shift of the North Pole due to the supposed transverse motion of the core. When account is taken of this, the effect of core rotation about a transverse axis appears to be about 1500 times greater than that actually observed from astronomical observation. Hence, the coupling between core

and mantle must be weak, or the motions deduced from geomagnetism may be faulty.

#### EQUATORIAL ELECTROJET EFFECTS DURING A GEOMAGNETIC BAY

E. H. VESTINE

Previous studies of the equatorial electrojet effects noted in the geomagnetic field at Huancayo, Peru, have been largely confined to those of the quiet-day diurnal variation, the disturbance daily variation, and the sudden commencement and initial phase of magnetic storms. It also appears that the electric currents overhead at Huancayo are sometimes enhanced during geomagnetic bays, which often appear strongly in the polar regions. Several possible cases of abnormal enhancement of field at the magnetic equator have been found, and it seems likely that the effect is more pronounced when the westward-directed electrojet at the auroral zone appears there abnormally late in local time during the morning. The best example found was the bay of November 15, 1922, where the augmentation at Huancayo amounts to over 100 gammas.

#### LABORATORY PHYSICS

##### NUCLEAR PHYSICS

N. P. HEYDENBURG AND G. M. TEMMER

##### COULOMB EXCITATION STUDIES

We have continued our studies of the rotational level systems occurring in the rare-earth nuclei, and have extended our survey of first-excited states of even-even nuclei. Several additional odd-mass nuclei have also been investigated.

A summary of our results on the study of nuclear energy levels in the rare-earth region appeared in last year's annual report. Ten nuclei were listed which exhibited a rotational level system having energies in good agreement with the strong-coupling theory of Bohr and Motelson. According to this theory, the first and second rotational levels should have

spins  $I_0 + 1$  and  $I_0 + 2$ , where  $I_0$  is the spin of the ground state. The energy of these levels is given by:

$$E = \frac{\hbar^2}{2\mathfrak{I}} [I(I+1) - I_0(I_0+1)]$$

where  $I$  is the spin of the level, and  $\mathfrak{I}$  is the effective moment of inertia of the nucleus. If the spin assignments for these levels are correctly given by the theory, it is to be expected that, in addition to gamma radiation from each level to the ground state, the cascade gamma ray from the second- to the first-excited state should be observed. Furthermore, coincidences should be observed when the second-excited state decays by way of the first-excited state with emission of two gamma rays in cascade. Since these low-energy



transitions are highly converted (i.e., de-excitation takes place by emission of an orbital electron instead of by a gamma ray), coincidences should also be observed with the  $K$  x-ray which follows the emission of a  $K$  conversion electron. Such  $K$ - $\gamma$  coincidences were indeed detected along with the  $\gamma$ - $\gamma$  coincidences from unconverted cascade transitions, in eight odd- $A$  rare-earth nuclei. A careful analysis of our earlier gamma-ray spectra also revealed the presence of the cascade gamma rays at the expected energy for the same eight rare-earth nuclei.

Having a value for this mixing ratio and a value for the nuclear magnetic moment in the ground state, one can separately compute the contributions to the gyromagnetic ratio from the odd particle ( $g_O$ ) and from the aggregate of particles ( $g_R$ ) in the deformed nucleus. The results of such calculations are given in table 11.

Several predictions that we made last year about the level schemes of specific nuclei have turned out to be correct. We reported a single gamma ray from each of the gadolinium isotopes of odd-mass numbers 155 and 157, and speculated that

TABLE 11

COULOMB EXCITATION RESULTS ON INTRINSIC AND COLLECTIVE  $g$  FACTORS FOR STRONGLY DEFORMED ODD- $A$  NUCLEI

Nucleus	$I_0$	$\mu_0$ (n.m.)	$\Delta E$ (kev)	$Q_0$ (barns)	$\delta^2$ *	$g_O$ †	$g_R$ ‡
$^{153}_{63}\text{Eu}$ .....	5/2	+1.6	105 ‡	7.4	0.38	0.72	0.45
$^{159}_{65}\text{Tb}$ .....	3/2	+1.5	79 ‡	8.7	0.013	1.70	0.0 §
$^{165}_{67}\text{Ho}$ .....	7/2	+3.3	112 ‡	7.3	0.039	1.10	0.39
$^{175}_{71}\text{Lu}$ .....	7/2	+2.9	136 ‡	8.0	0.13	0.92	0.50
$^{177}_{72}\text{Hf}$ .....	7/2	+0.61	138 ‡	8.0	1.64	0.21	0.06
$^{179}_{72}\text{Hf}$ .....	9/2	-0.47	141 ‡	6.9	0.15	-0.04	-0.40

\* Intensity ratio  $E2/M1$  for stop-over transition.

† Because of ambiguity in the sign of  $g_O - g_R$ , there exists another pair of solutions in all cases. We have arbitrarily selected those yielding the smaller value of  $g_R$  ( $Q_0/\delta > 0$ ). There seems to be some correlation between the size of  $g_R$  in relation to  $Z/A$  and the nature of the odd particle (proton vs. neutron).

‡ Stop-over transition.

§ Value extremely sensitive to  $\delta^2$ .

In the Coulomb excitation process, only electric quadrupole ( $E2$ ) transitions are induced; however, once a level is excited it will decay by all allowed radiation components. Hence, according to the selection rules, the second-excited state should decay to the ground state by a pure  $E2$  transition. It can also decay to the first-excited state by a mixture of  $E2$  and  $M1$  (magnetic dipole) radiation. From the relative intensities of the crossover (from second to ground state) and the cascade gamma rays, and assuming the theoretical ratio for the  $E2$  radiation components in these transitions as given by Bohr and Mottelson, one can compute the mixing ratio for  $E2$  and  $M1$  radiation in the cascade transition.

in both cases the observed gamma rays might be due to the de-excitation of the second rotational levels, since their intensities and energies corresponded closely to those observed from second rotational levels in neighboring nuclei having well defined rotational systems. The gamma radiation due to the first-excited states should then have occurred at an energy of about 60 kev, too close to the x-ray line of gadolinium to be clearly resolved. Such a low-energy transition would also be strongly converted. Recently the conversion electron spectra corresponding to the predicted first-excited states in the odd- $A$  gadolinium isotopes have been observed by groups working at Duke University and

Copenhagen. We have observed coincidences between the cascade transitions and  $K$  x-rays in each of these isotopes. We have also confirmed the presence of the cascade gamma rays from the second excited states of these isotopes by using enriched targets of  $\text{Gd}^{156}$ ,  $\text{Gd}^{158}$ , and  $\text{Gd}^{160}$ . The cascades in the enriched odd isotopes were partly masked by transitions of nearly the same energy from those even nuclei which still constitute about 40 per cent of the enriched target material.

A recent redetermination of the ground-state spins of the two odd hafnium isotopes, giving the values  $7/2^-$  for  $\text{Hf}^{177}$  and  $9/2^+$  for  $\text{Hf}^{179}$ , are in excellent agreement with the spin values we predicted from our observed energy ratio of the rotational states.

From our Coulomb excitation results on natural europium, which has about equal amounts of  $\text{Eu}^{151}$  and  $\text{Eu}^{153}$ , we had assumed that all but one of the observed gamma rays were due to  $\text{Eu}^{153}$ . When enriched isotopes recently became available, we found that  $\text{Eu}^{151}$  had two gamma rays at 110 kev and 195 kev which had nearly the same energies as two much stronger gamma rays from  $\text{Eu}^{153}$ . By coincidence measurements the level schemes shown in figure 17 were established for  $\text{Eu}^{151}$  and  $\text{Eu}^{153}$ . The contrast in the level schemes for these two isotopes is illustrated in figure 17.  $\text{Eu}^{153}$  has a well behaved rotational level system, whereas neither the level spacings nor the intensities of the transitions of  $\text{Eu}^{151}$  correspond to a rotational band. This result is further evidence for the marked break in the systematics of nuclei between neutron numbers 88 and 90. This break is also shown in the first-excited state energies and transition rates of the even-even isotopes of neodymium, samarium, and gadolinium, as is illustrated in figures 17 and 18.

Nuclei with neutron numbers  $N \geq 90$ ,  $N \leq 112$  are strongly deformed, and exhibit the rotational energy states predicted

by the strong-coupling approximation of the unified model. The even-even nuclei in this region have the predicted ratio of second- to first-excited state energy of 3.3. For nuclei with  $N \leq 88$  this ratio becomes more nearly 2.2, and the simple strong-coupling approximation is no longer applicable. Various theoretical attempts have been made to explain the systematic behavior in even-even nuclei, illustrated in figure 19, as well as the fact that the transi-

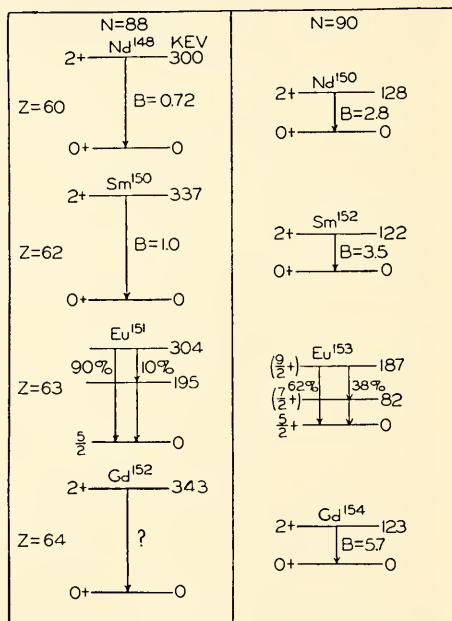


FIG. 17. Level schemes for nuclei with neutron numbers  $N = 88$  and  $90$ , illustrating the break in the systematics between these two neutron numbers.

tions in this region are still from 10 to 30 times stronger than would be expected for a single particle. Our understanding of the nuclei in this region of mass numbers has not as yet reached the level of the heavier (rare-earth) nuclei, especially as far as the odd- $A$  nuclei are concerned.

The systematics of the first-excited states of even-even nuclei with neutron numbers from  $N = 86$  to  $N = 146$  are illustrated in figure 18. Both  $\beta_B^2$  and  $\beta_B^2$  have been

plotted on one graph, where the quantities  $\beta$  come from the strong-coupling theory and are given by:

$$\beta_E^2 = 2.43 \times 10^5 / A^{5/3} \Delta E \text{ (kev)}$$

$$\beta_B^2 = 8.48 \times 10^4 B(E_2) / Z^2 A^{4/3}$$

$\beta_E^2$  is inversely proportional to the first-excited state energy  $\Delta E$ , and  $\beta_B^2$  is proportional to the transition probability  $B(E_2)$

We have also observed energy levels in the enriched isotopes of  $\text{Cu}^{63}$ ,  $\text{Cu}^{65}$ , and  $\text{V}^{50}$ . The levels observed in  $\text{Cu}^{63}$  were reported last year but are now definitely established as due to this isotope. In  $\text{Cu}^{65}$  we observed two gamma rays at 815 kev and 1150 kev.  $\text{V}^{50}$  is of interest in that it is a nucleus having odd  $N$  and odd  $Z$ . It is the only enriched odd-odd nucleus in

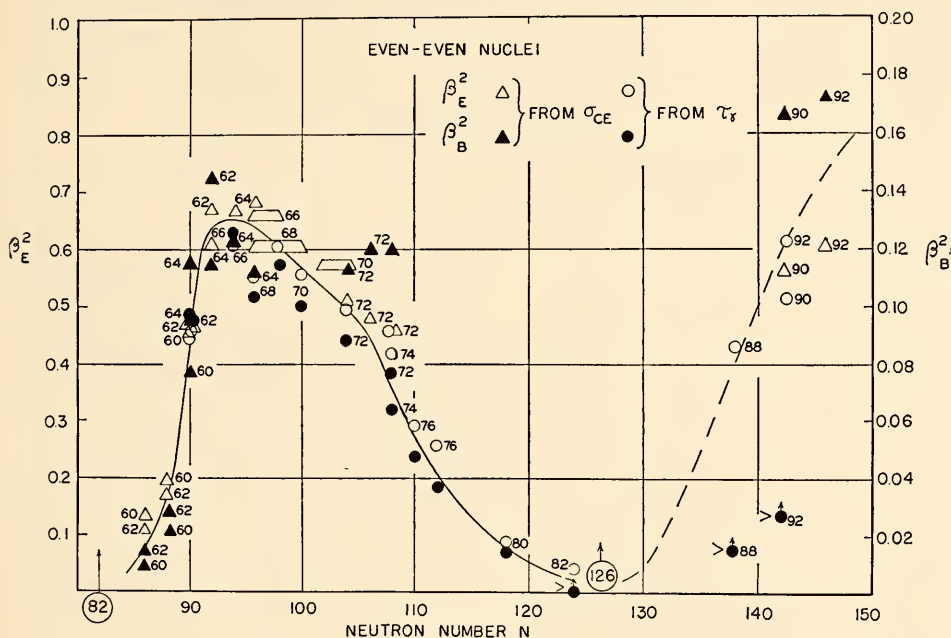


FIG. 18. Summary of Coulomb excitation results for deformed, even-even rare-earth nuclei. Empty symbols represent "deformations" obtained from position of first-excited  $2^+$  states and have ordinates inversely proportional to  $\Delta E$ ; full symbols represent "deformations" obtained from reduced quadrupole transition probabilities to the  $2^+$  states, and have ordinates proportional to  $B(E_2)$ . Atomic numbers of various elements are given in the diagram. Circles refer to results from lifetime and resonance fluorescence measurements as summarized by Sunyar.

(expressed in  $10^{-48}$  cm) deduced from the gamma-ray intensities. It can be seen that there is good correlation between these two quantities: both become large when far removed from the closed shells at  $N=82$  and  $N=126$ . This is the region of nuclei with large deformations.

A similar graph for  $N < 82$  is shown in figure 19. Again, a correlation between the values of  $\beta_E^2$  and  $\beta_B^2$  can be seen in this region.

the medium and heavy  $A$  region which is now available. By Coulomb excitation with 3-Mev alpha particles we observe a 225-kev gamma ray which probably corresponds to the first-excited state of  $\text{V}^{50}$ .

We have investigated the rare gases xenon and krypton by Coulomb excitation with 6.4-Mev alpha particles by using gas targets. Gamma rays were observed and tentatively assigned to the nuclei on the basis of abundance, systematics, and levels

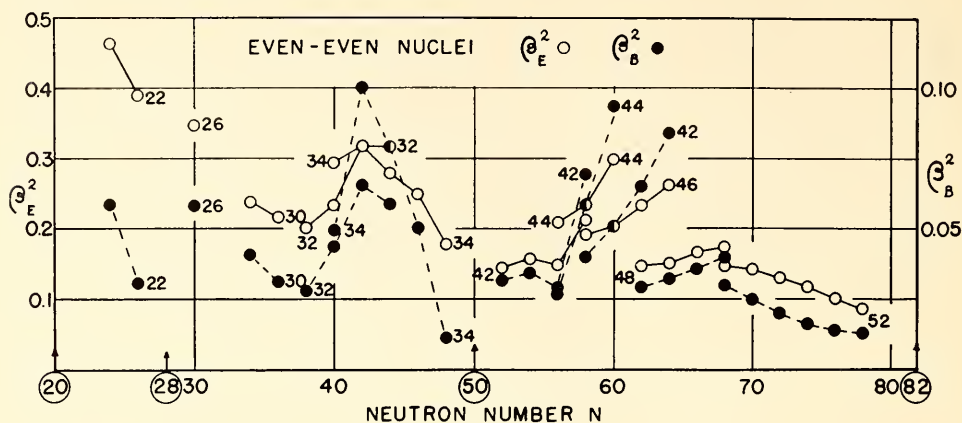


FIG. 19. Summary of Coulomb excitation results for medium-heavy even-even nuclei. Definitions similar to those in legend of figure 18.

known from beta-decay studies, as shown in table 12.

#### ALPHA-ALPHA SCATTERING

We have recently made some revisions in the analysis of our  $\alpha$ - $\alpha$  scattering results which were reported in Year Book No. 52 (1952-1953). A phase-shift analysis of our differential cross-section curves from 400-kev to 3.00-Mev alpha particles gives the following results: Above 400 kev the nuclear  $s$ -wave interaction begins to contribute, starting at a phase shift  $K_0$  near  $\pi$ , and smoothly decreasing with increasing energy to about  $128^\circ$  at 3 Mev. Starting at 2.5 Mev, a small *positive*  $d$ -wave phase shift,  $K_2$ , is found necessary to ac-

count for the observed angular distributions, reaching a value of  $2.5^\circ$  at 3 Mev. Absolute values of the cross sections were determined by fitting the relative angular distributions with the single parameter  $K_0$  below 2 Mev, and by comparison with Rutherford scattering in argon above 2 Mev. A summary plot of all angular distribution data in terms of ratio to Mott scattering is given in figure 20, where the solid curves represent the best theoretical fits we obtained from a phase-shift analysis. Figure 21 shows a plot of the phase shifts  $K_0$  and  $K_2$  as a function of the energy. Recent  $\alpha$ - $\alpha$  scattering work at the Rice Institute for  $\alpha$  energies in the range 3 to 6 Mev definitely showed the  $d$ -wave

TABLE 12  
TENTATIVE TRANSITIONS IN KRYPTON AND XENON

Element	$A$	Relative abundance (per cent)	$E_\gamma$ (kev)	$I_0 - I^*$
$_{36}\text{Kr}$ .....	80	2.3	610	$0^+ - 2^+$
	82	11.5	775	$0^+ - 2^+$
	83	11.5	457	$9/2^+ - ?^+$
	84	57.0	880	$0^+ - 2^+$
$_{54}\text{Xe}$ .....	130	4.1	530	$0^+ - 2^+$
	129	47.6	286,364	$\{1/2^+ - ?^+$
	131			$3/2^+ - ?^+$
	132	26.9	670	$0^+ - 2^+$
	134	10.4	870	$0^+ - 2^+$



resonance associated with the first-excited state of  $\text{Be}^8$  at  $\sim 3$  Mev, thus establishing the spin of the latter as  $2^+$ . Our careful survey of the low-energy region (below

upper limit of  $4 \times 10^{-15}$  sec, this locates the lifetime within a factor of 20.

It is possible to account for all low-energy features of the  $\alpha$ - $\alpha$  interaction with a rather simple two-body potential even though the alpha particle is a complex structure in itself.

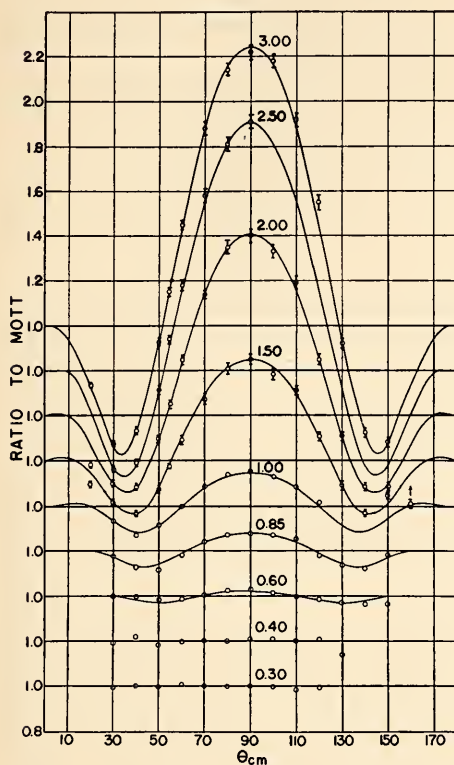


FIG. 20. Summary of angular distributions for  $\alpha$ - $\alpha$  scattering in the energy range 300 kev to 3.00 Mev. Ratio of observed to Mott differential cross sections is plotted against center-of-mass scattering angle  $\theta$ . Values at center of curves refer to bombarding energy in Mev. Vertical scale shown correctly for the highest curve (3 Mev), but arbitrarily displaced by 0.20 unit downward for 2.5 Mev, 0.40 unit for 2.0 Mev, etc., to avoid superposition. The value 1.0 belonging to each energy is indicated to the left. Curves, symmetrical about  $90^\circ$ , are *theoretical*, obtained by phase-shift analysis of the data, using values for  $s$ -wave and  $d$ -wave phase shifts  $K_0$  and  $K_2$  shown in figure 21.

400 kev) containing the ground state of  $\text{Be}^8$ , and the absence of any measurable effect, leads to a *lower* limit for the mean life of the ground state of  $\text{Be}^8$  of  $2 \times 10^{-16}$  sec. Combined with a recent established

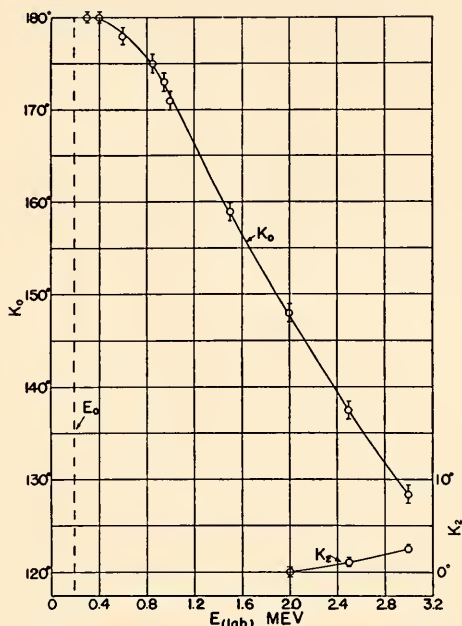


FIG. 21. Dependence of phase shifts for  $\alpha$ - $\alpha$  scattering on energy. Left-hand scale applies to  $s$ -wave phase shift  $K_0$ ; right-hand scale applies to  $d$ -wave phase shift  $K_2$ . Dotted vertical line marked  $E_0$  at 189 kev represents probable behavior of  $K_0$  upon going through the resonance corresponding to the ground state of  $\text{Be}^8$ .

#### INSTRUMENTATION

*Electrostatic generator.* During the year the replacement of the old accelerating tube in the pressurized electrostatic generator has been completed. The new tube is similar in design to the tube used by Herb at Wisconsin. It is made up of 3-inch porcelain sections, with re-entrant spun-aluminum electrodes. The sections are cemented together with a cold-setting plastic into convenient, platform-length subassemblies which could then be bolted together in place. After an outgassing

period the machine has operated up to 4.25 Mev, and stable operation has been achieved for long periods at 4.1 Mev. This is a considerable improvement over the old tube, which would not operate well above 3 Mev.

A precision electrostatic analyzer for the charged-particle beam of the high-voltage generator is nearing completion, and will soon be ready for installation and testing.

#### CYCLOTRON

Two quadrupole focusing magnets have been completed for the cyclotron. These are now being installed, and tests for the focusing of an external beam are in progress.

#### BIOPHYSICS

E. T. BOLTON, R. J. BRITTEN, D. B. COWIE,  
E. H. CREASER, AND R. B. ROBERTS

A renewed attempt is being made by biophysicists to define biophysics, and to determine whether there is an area of mutual interest to all those who call themselves by the name. Symptomatic of this self-examination are the several meetings to form a national society. As might be expected, no universally accepted definition has emerged; each biophysicist must still define his own concept of biophysics. The biophysics section of our Department long ago realized that there is no particular area of biology that can properly be described as the province of biophysics. We believe that biophysics is defined solely by what a biophysicist thinks about and does. A biophysicist is one who applies his training in physics to the problems of biology.

This conviction is largely the result of the history and experience of the section. About nine years ago, a small group trained primarily in nuclear physics was invited to see what contributions it might make to the problems of biology. In the following years we have worked closely with biologists, physiologists, biochemists, and other specialists. We have found no

formula that would distinguish our interests from theirs. The only consistent difference among us has been the difference in training.

From the beginning we have been concerned primarily with the problems of growth and reproduction, which, at the molecular level, are the problems of protein and nucleic acid synthesis. We have attempted to study these processes in intact cells growing exponentially in synthetic media. In such a system interactions among different types of cells are minimized. Direct approaches to these problems are exceedingly difficult. In a complicated jigsaw puzzle it is often best to begin by filling in the edges. The edges may have less interest than the central picture, but they provide a framework and may furnish ways to approach the center. Thus, for several years we studied the synthesis of the small molecules—amino acids, purines, and pyrimidines—that are the building blocks of proteins and nucleic acids. In the last two years we have turned to the study of the incorporation of these molecules. In general, small molecules are first concentrated by the cells into a relatively loosely held “pool” and subsequently linked into the macromolecules. The past year’s work has greatly increased our knowledge of the characteristics of these pools. In addition, some of the immediate precursors of nucleic acid have been determined, and an upper limit of 3 seconds for the time required for protein synthesis has been established.

Most of the work covered by this report concerns *Escherichia coli*. It should be emphasized, however, that the object is not to study *E. coli* as such but to find general concepts from experiments that happen to be most easily carried out with *E. coli*. Parallel work has been done with *Torulopsis utilis*, a yeast-like organism. The differences between the two organisms are often instructive, and show the limits of generality of concepts developed from experiments on either one. For example, the metabolic pools of amino acids are very

much larger in *T. utilis* and more difficult to extract. Some work has been done with *Hydra littoralis* to determine whether tracer studies of the type carried out in the single-celled organisms can be extended to simple multiple-celled animals. Finally, Dr. L. B. Flexner, a Research Associate of the Carnegie Institution, has successfully followed  $C^{14}$  glucose and amino acid tracers from a subcutaneous injection through the blood into cellular pools of free amino acids and eventually into the protein of newborn mice.

#### ESCHERICHIA COLI

Various avenues of approach have been followed in the work with *E. coli*; all of them converge toward the central problems of macromolecular synthesis. At the end of the last report year it had been established that *E. coli* concentrates amino acids from its surrounding fluid into what may be termed a metabolic pool within the cell. Amino acids required for protein synthesis are not taken directly from the medium but are drawn from the pool. It is therefore clear that the pool and the mechanism for forming the pool are closely related to the mechanisms of linking together the amino acids in the proper sequence to form a polypeptide chain of a protein. While still in the pool the amino acids may be extracted by a number of mild agents, and chromatography has failed to reveal any chemical alteration. From the evidence then available it appeared that the observed concentration within the cells might be explained in terms of simple adsorption. An alternative explanation, favored by other groups, in terms of a mechanism whereby the amino acids were pumped into a semipermeable region, could not be ruled out.

A large share of this year's work has been directed toward obtaining further information about this metabolic pool, particularly any facts that might rule out one or the other model. Experiments have been carried out to measure the maximum size of the pools, and the rates of exchange be-

tween the pool material and the medium. Other experiments have explored the ways in which one amino acid can prevent the concentration of a second, similar amino acid even though one does not substitute for another in the formation of proteins. Information of a different kind has come (a) from one set of experiments which shows that the mechanism holding the amino acids within the cell is sensitive to the osmotic strength of the medium, and (b) from another set designed to measure the volume within the cell which is accessible to amino acids by free diffusion. These experiments taken as a whole seem to rule out both of the simple models, adsorption or pump, and apparently indicate that the amino acids may be trapped within the framework of ordered structures of the cell, possibly the microsomes.

The studies of metabolic pools have been extended to include the purine and pyrimidine bases that provide the building blocks of nucleic acid, and the phosphorus compounds which are related both to nucleic acid synthesis and to energy transfer.

Several special lines of attack have been followed to answer particular questions. Thus the properties of cell fragments have been investigated in an effort to find a system simpler than a whole cell which would carry out some of the synthetic reactions. The nucleic acid formed during the period when the cells were adapting to a new carbon source was fractionated to see whether a special kind of nucleic acid could be found when a special protein was made. The action of the drug 6-mercaptopurine was studied to determine whether this drug would produce a situation (opposite to that observed with chloramphenicol) in which protein was synthesized although nucleic acid synthesis was blocked.

Many of these experiments were made possible by the filter technique developed last year. Samples of the cells can be removed from the medium simply by passing the suspension through a special filter. Other samples can be extracted,



usually by mixing with trichloroacetic acid, and then filtered. In this way it is possible to make rapid kinetic studies of the incorporation of various tracer-labeled compounds, observing both the rate at which metabolic pools are formed and the rate at which the pool material is passed on into the macromolecules. The details of the experiments are given below, and their significance is discussed.

### Amino Acid Incorporation

#### Pool size as a function of concentration.

The size of the pool formed from proline over a wide range of concentrations has been studied. Figure 22 is a plot of the

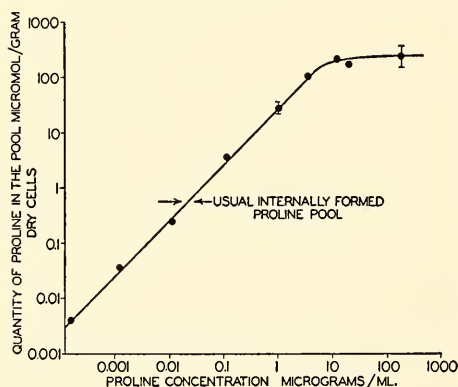


FIG. 22. Quantity of  $C^{14}$  proline taken up into the pool as a function of the concentration of  $C^{14}$  proline added to a growing culture of *E. coli*.

pool size versus the concentration of the proline supplied. The long linear part of this curve which extends over a concentration range of nearly  $10^5$  corresponds to an internal concentration, per milliliter of cells, which is 500 times the concentration in the medium. The pool appears to reach a maximum or saturation value of about 250 micromoles per gram dry cells. This is an impressively large figure, corresponding to an average concentration of 0.06 molar within the cells. The saturation value for valine appears to be somewhat lower (60  $\mu\text{mol/gr dry}$ ), and the shape of the curve appears to be different, al-

though there is a range of concentration over which it is linear with about the same ratio of internal to external concentration as in the case of proline.

**Valine incorporation at low concentrations.** When valine is supplied at low concentrations the uptake is very rapid, and corresponds to the removal of all the valine from 600 cell volumes of medium per second by each cell. An approximate calculation of the diffusion of valine into a sphere of volume equal to the cell volume, assuming the concentration at the surface of the sphere to be zero, gives a flow equivalent to 5000 cell volumes per second. Thus the cell is capable of taking up valine at a rate approaching the maximum set by diffusion toward the cell.

**Exchange and energy requirement.** Last year's work showed that an energy source was required to build up a pool by concentrating amino acids from the medium. Once the pool is formed, however, no exogenous energy supply is needed to maintain it or to permit exchange between the pool and the medium. When an amino acid is supplied to cells that have exhausted the supply of glucose, it enters the pool at less than 5 per cent of the normal rate observed in the presence of glucose. This low rate is presumably due to endogenous reserves of energy.

In contrast, when cells supplied with an amino acid and glucose have already built up a pool, the pool remains constant after the glucose is exhausted. After the glucose is exhausted, exchange can occur between the amino acid molecules in the medium and those in the pool. These results are shown in figure 23. In both experiments, a relatively large amount of nonradioactive proline and a limited supply of glucose were added.  $C^{14}$  proline was added to one of the cultures at time zero (solid line) and to the other at 13 minutes (dotted lines). The solid curves show that the pool size does not increase when glucose is exhausted, and the dotted curves show that exogenous proline enters the pool. It is clear, therefore, that exchange occurs be-



tween the proline in the pool and the exogenous proline in the absence of an energy source.

In one type of experiment, growing cells were allowed to exhaust the glucose supply, and then very high specific activity  $C^{14}$  glucose was added in an amount sufficient to support growth for only a few minutes. Seventy per cent of the radioactivity incorporated quickly appeared in the trichloroacetic acid (TCA)-soluble fraction and remained there without significant change for several hours. After one-half hour, samples of the TCA-soluble pool and the medium were withdrawn and analyzed

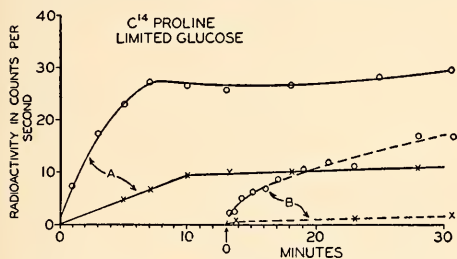


FIG. 23. Maintenance and exchange of pool proline in the absence of glucose. In both experiments, growing cells were suspended at time zero in medium containing 10  $\gamma$ /ml glucose and 0.87  $\gamma$ /ml  $C^{12}$  proline. For curve A a small quantity of  $C^{14}$  proline was added at time zero. For curve B an equal quantity of  $C^{14}$  proline was added at 13 minutes. In each case the upper curve, O, represents the total  $C^{14}$  proline taken up, and the lower curve, x, the  $C^{14}$  proline incorporated into the pool. The difference is the  $C^{14}$  proline in the pool.

by chromatography. Large quantities of amino acids were found in the pool and traces in the medium. The ratios of the concentrations of the amino acids in the cells to the concentrations in the medium were evaluated; they ranged from 28,000 for valine, 14,000 for glutamate, 7300 for proline to 2300 for aspartic acid. Again, it is clearly shown that the cell has the capacity in the absence of glucose to maintain a highly concentrated metabolic pool.

Exchange is also an important process in the presence of glucose. An example is shown in figure 24.  $C^{12}$  valine at 0.3

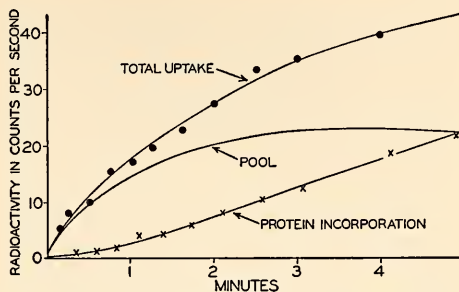


FIG. 24. Demonstration of exchange during pool formation. Growing cells (0.07 mg dry/ml) were supplied 0.3  $\gamma$ /ml of  $C^{14}$  valine 4 minutes before the addition of the  $C^{14}$  valine. At the time  $C^{14}$  valine was added, the unlabeled pool had reached a steady state. The rapid initial incorporation of  $C^{14}$  valine shows that exchange is occurring between pool valine and external valine.

$\gamma$ /ml was added 4 minutes before time zero. This time is sufficient to allow the pool to come up nearly to its steady-state value. At time zero a small quantity of valine of very high specific radioactivity was added. If there were no exchange the total curve would rise linearly from the origin at its final rate, since proline molecules could enter the pool only at the rate at which they were withdrawn from it for protein synthesis. In fact, it rises initially much more rapidly. Figure 25 shows schematically the flow rates calculated from the experiment of figure 24.

Similar experiments, carried out over a wide range of concentrations, indicate that exchange is observable except at the very lowest concentrations, and that the magnitude of the exchange flow is greater at higher concentrations. Pools formed in-

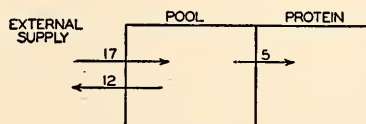


FIG. 25. Schematic diagram showing the exchange of pool valine during incorporation at a concentration of 0.3  $\gamma$ /ml. The numbers are flow rates in micromoles per gram dry cells per 100 sec, calculated from the curve shown in figure 24.

ternally from glucose presumably also exhibit exchange with the small quantities of amino acids in the medium. The concentrations present in the medium are so small under these conditions, however, that a direct demonstration of exchange is difficult.

*Interactions among valine, leucine, and isoleucine.* Further information about the pool and its formation can be obtained by studies of the interactions of similar amino acids. The interactions of leucine, isoleucine, and valine have been examined

protein decreases to a steady-state value one-thirtieth of the control.

Figure 28 shows the results of an initially identical experiment in which an equally large quantity of  $C^{12}$  leucine was added at 40 seconds. The  $C^{14}$  isoleucine is removed from the pool by exchange with the leucine. During the 40 seconds after addition, while a measurable quantity of isoleucine remains in the pool, the rate of isoleucine incorporation into the protein is unaffected. After this period, when the isoleucine pool has dropped to a very

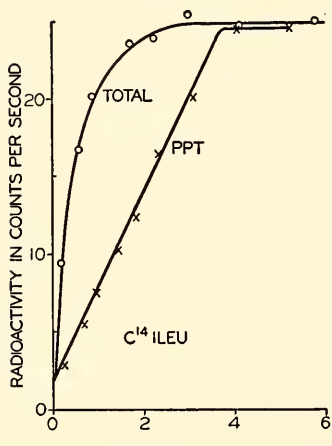


FIG. 26. Leucine-isoleucine interaction; control: 0.3  $\gamma$ /ml  $C^{14}$  isoleucine added at time zero to 0.25 mg dry/ml of growing cells.

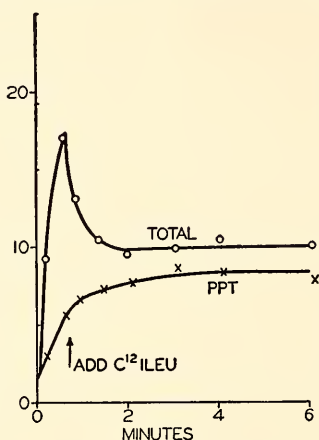


FIG. 27. Leucine-isoleucine interaction; effect of carrier isoleucine. Same as figure 26 with 10  $\gamma$ /ml  $C^{12}$  isoleucine added at 40 seconds.

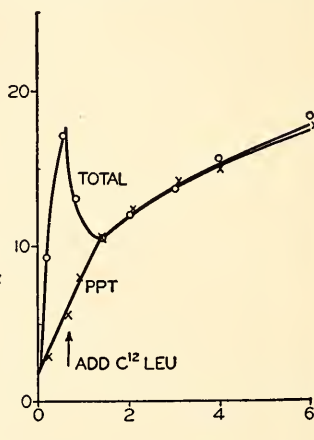


FIG. 28. Leucine-isoleucine interaction; effect of leucine competitor. Same as figure 26 with 10  $\gamma$ /ml  $C^{12}$  leucine added at 40 seconds.

closely, because these three amino acids compete with one another for entry into the pool.

Figure 26 shows the uptake of  $C^{14}$  isoleucine, as control. Figure 27 shows the results of an initially identical experiment in which 30 times the original quantity of  $C^{12}$  isoleucine was added at 40 seconds. With this concentration change the pool does not increase in proportion to the concentration, and  $C^{14}$  isoleucine is removed from the pool by exchange. The specific radioactivity of the pool isoleucine immediately starts to decrease, and as a result the rate of incorporation of  $C^{14}$  isoleucine into the

small value, the incorporation into protein continues at about one-sixth the rate of the control. Similar experiments have been performed for various combinations of the three compounds valine, leucine, and isoleucine. In each case the rate of incorporation into protein of the labeled compound from the pool is unaffected until the quantity in the pool drops to a low value. The degree of suppression of the steady-state pool and rate of protein incorporation are different in the various cases, as is described below.

In order to explore the interactions among several different compounds over

a range of concentrations a somewhat simpler experimental design has been used. An example is shown in figure 29, in which the labeled compound and an unlabeled competitor were added to a growing culture at the start of the experiment. Table 13 summarizes the results of a number of similar experiments.

These experimental findings lead to conclusions of consequence for understanding the nature and function of the pool.

(a) Some of the mechanisms for pool formation are less selective than those for the incorporation of an amino acid into protein. The substitution of any amino acid residue of the protein by another has

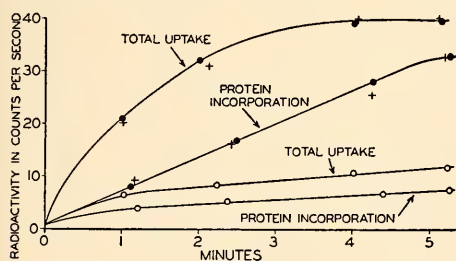


FIG. 29. Typical experiment from which the data of table 13 were obtained. For all the curves, 0.3  $\gamma$ /ml  $C^{14}$  valine was added to 0.08 mg dry/ml growing cells at time zero. For the control  $\bullet$ , no competitor was present. For the curves marked +, 10  $\gamma$ /ml *d*-valine was added at time zero. For the curves marked  $\circ$ , 10  $\gamma$ /ml norvaline was added at time zero.

not been observed in a wide variety of tests in which a 10 per cent substitution would have been detectable.

(b) The rate of protein incorporation of an amino acid present in a sizable quantity in the pool is not in general affected by large quantities of another amino acid in the pool. An apparent exception is shown in lines 24, 25, 26, and 27 of table 13. The compound  $\alpha$ -ketovalerate does not suppress the valine pool but suppresses the rate of incorporation of  $C^{14}$  valine by 65 per cent. This compound, however, may simply be converted to valine and thus dilute the specific radioactivity of the pool valine.

(c) One amino acid may suppress the entry of another amino acid into the pool, but no case has been observed of a pool amino acid's being prevented from leaving the cell by a second amino acid. The inference is that if a transport mechanism exists for entry into the pool the same mechanism is not utilized for exit.

(d) A simple reciprocity does not occur in the interaction of isoleucine and valine. As shown in lines 20 and 3 of table 13, the effect of isoleucine on valine is greater than that of valine on isoleucine. Line 16, however, shows that an equal quantity of isoleucine depresses the valine pool by only 30 per cent.

(e) Isoleucine acts, in effect, as a "dog in the manger" in suppressing the valine pool. Line 17 of table 13 shows that when isoleucine is present at twice the valine concentration the pool is suppressed to one-half its normal value at this concentration. In this concentration range, however, if the valine concentration is tripled the pool is tripled. Indeed, the maximum pool size for either valine or leucine is at least ten times the pool size at the concentration used. This result may not be explained by simple enzyme-site competition models, whether the sites are considered to be part of a transport mechanism or the actual mechanism for holding the amino acid in the pool.

(f) An amino acid may be incorporated into the protein at a good fraction of the control rate, as shown after 2 minutes in figure 28, even though the pool is so small that it cannot be measured.

*Effect of osmotic strength on the pool.* A series of exploratory experiments have been carried out on the effect of salt concentration and osmotic strength on the maintenance of a preformed pool and the ability of the cells to form amino acid pools. Low salt concentrations remove the pool, as is indicated in figure 30. For example, if the cells are washed with a one-tenth dilution of the usual C medium, 85 per cent of the pool will be removed. If they are washed in water (even for so

short a period as 15 seconds), all the pool will be removed.

The evidence shows that it is the osmotic and not the ionic strength of the washing medium that influences the pool.

osmotic strength. When washes are performed with 0.37 osmolal solutions of glucose, sucrose, glycine, or sodium chloride, the pool is unaffected. Solutions of glycerol, urea, or ethanol of the same osmotic

TABLE 13

SUMMARY OF THE INTERACTIONS OF ISOLEUCINE, LEUCINE, VALINE, AND RELATED COMPOUNDS DURING POOL FORMATION \*

No.	Labeled compound	Conc. ( $\gamma$ /ml)	Competitor	Conc. ( $\gamma$ /ml)	Per cent suppression of pool	Per cent suppression in rate of incorporation into protein
1	Isoleucine	0.29	Leucine	8.8	98	75
2	Isoleucine	.17	Leucine	9.1	98	82
3	Isoleucine	.29	Valine	8.8	91	75
4	Isoleucine	.17	Valine	9.1	94	75
5	Leucine	.02	Valine	3.6	> 60	..
6	Leucine	.29	Valine	10.	86	10
7	Leucine	.29	Valine	10.	90	35
8	Leucine	.02	Isoleucine	0.29	0	0
9	Leucine	.29	Isoleucine	10.	95	45
10	Leucine	.32	Norleucine †	9.6	0	0
11	Leucine	.32	Norvaline †	9.6	67	58
12	Valine	.30	Leucine	9.1	80	73
13	Valine	.007	Isoleucine	0.3	90	92
14	Valine	.02	Isoleucine	10.	94	> 95
15	Valine	.15	Isoleucine	0.075	16	20
16	Valine	.15	Isoleucine	0.15	32	39
17	Valine	.15	Isoleucine	0.30	54	54
18	Valine	.15	Isoleucine	1.5	85	89
19	Valine	.10	Isoleucine	4.0	87	90
20	Valine	.30	Isoleucine	10.	> 90	> 95
21	Valine	.30	d-Valine	10.	0	0
22	Valine	.30	Norleucine †	10.	65	0
23	Valine	.30	Norvaline †	10.	84	84
24	Valine	.29	$\alpha$ -Ketoisovalerate	0.29	+ 18 ‡	30
25	Valine	.29	$\alpha$ -Ketoisovalerate	0.87	+ 35 ‡	45
26	Valine	.29	$\alpha$ -Ketoisovalerate	2.9	+ 65 ‡	58
27	Valine	0.30	$\alpha$ -Ketoisovalerate	7.5	..	65

\* The values for the suppression of the pool and reduction of the rate of incorporation of radioactivity into the protein are calculated from individual experiments such as those shown in figure 29.

† Norvaline at 10  $\gamma$ /ml suppresses growth rate by 42 per cent; norleucine at 10  $\gamma$ /ml does not suppress growth rate.

‡ Increase in pool size.

If glucose is added in place of the salts of the standard medium to maintain the original osmotic strength (0.37 osmolal), the pool will not be removed. Also, if various concentrations of glucose are used as washes, the amount removed is about the same as that removed with salt of the same

strength, however, remove nearly all the pool. About half of the pool is removed with 0.37 osmolal sodium acetate (adjusted to pH 7). Thus it appears that one aspect of the osmotically sensitive structure may be explored by means of the response to different solutes. Washing with glucose



solutions of higher osmolality than 0.37 has little effect on the pool. Similar results have been obtained whether the tests were carried out on large proline pools (50  $\mu\text{mol/gr}$  dry cells), on small (1 to 10  $\mu\text{mol/gr}$  dry cells) proline or valine pools, or on the complex pool internally synthesized from  $\text{C}^{14}$  glucose.

Further experiments have shown that the pool can be re-formed in media of low osmotic strength, and the recovery is very rapid. Figure 31 shows the result of an experiment in which a suspension was di-

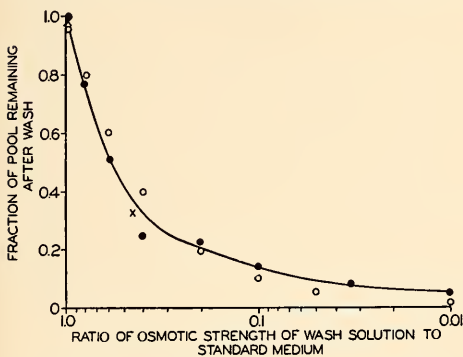


FIG. 30. Removal of pool by washing cells with solutions of low osmotic strength. Growing cells were supplied  $\text{C}^{14}$  proline and allowed to form a pool; then samples were collected on a membrane filter. Various wash solutions were passed through the cell pad on the filter. The points marked ● were washed with various dilutions of the standard growth medium; the points marked ○, with  $\text{NaCl}$  solutions; and the points marked x, with glucose solutions.

luted with 2 volumes of water while incorporation of proline was in process. At the instant of dilution the pool dropped sharply, and after 1 minute it started back toward its original value. In another experiment, cells were centrifuged from a growing culture, suspended in a large volume of water, recentrifuged, and suspended in C medium supplemented with  $\text{C}^{14}$  proline and a small amount of glucose. In this case the rate of pool formation was about one-half of the control even during the first minute. Some recovery may have occurred during the 10 minutes

required for centrifuging in the absence of salts or energy source. It is nevertheless clear that recovery of the pool-forming capacity after osmotic shock does not require resynthesis of any significant fraction of the cell material in order to repair damaged structures.

The results of a few experiments shown in table 14 indicate that the saturation pool size for proline is roughly proportional to the osmotic strength of the medium, and independent of the ionic strength.

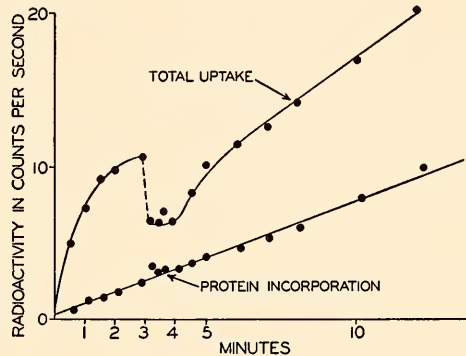


FIG. 31. Recovery of pool after removal due to reduction in osmotic strength. At time zero, 0.3  $\gamma/\text{ml}$   $\text{C}^{14}$  proline was added to 0.08 mg dry/ml of growing cells. At 3 minutes, 2 volumes of water containing 0.3  $\gamma/\text{ml}$   $\text{C}^{14}$  proline were added.

The striking osmotic effects aid in approaching an understanding of the process of pool formation and maintenance in *E. coli*: they explain the failure of both the extreme models that have been currently considered. One model suggests that the pool amino acids are held within a semi-permeable membrane ("bag") and that active transport mechanisms ("pumps") maintain the internal concentration. If features are added to this model in an effort to explain the rapid repair of the membrane after osmotic shock and the increase in capacity with increasing osmotic strength of the medium, it is changed beyond recognition.

The other model suggests that the amino acids are held on "sites" on the macro-

molecules of the cell by labile bonds. In order to preserve such a model it must be assumed that the "shape" or electron distribution of the site regions is dependent on the osmotic strength of the medium in certain special ways. For instance, the number of effective sites must depend on the osmotic strength of the medium.

Clearly, a deeper understanding of the internal macromolecular structure of the cell than is supplied by either of these

TABLE 14

SATURATION POOL SIZE AS A FUNCTION OF OSMOTIC STRENGTH OF THE MEDIUM \*

OSMOLALITY			POOL SIZE ( $\mu\text{mol/gr dry}$ )
Due to C medium	Due to sucrose	Total	
0.37	0.54	0.91	450
.04	.33	.37 †	300
.37	...	.37 †	217
.04	.17	.21	98
.20	...	.20	50
.04	.04	.08	29
.084	...	.084	46
0.05	...	0.05	6

\* Growing cells were centrifuged, resuspended at 0.25 mg dry/ml medium of the constitution shown in columns 1 and 2 with 0.2 per cent glucose and 24  $\gamma$ /ml  $\text{C}^{14}$  proline. After 18 minutes, samples were taken for measurement of the total uptake and the incorporation into protein. Other evidence indicates that the cells would recover from the osmotic shock and take up a proline pool approaching the saturation value in the time allowed, at this high concentration of proline.

† Osmotic strength of standard buffer (C medium).

models must be attained to explain the osmotic effects. In turn, these effects should aid in attaining such an understanding.

*Permeability.* A renewed interest on the part of biochemists in the permeability of the bacterial cell is becoming increasingly evident. In our own work, measurements of the permeability of *E. coli* have taken on a new importance since they contribute to the understanding of the mechanisms for the formation and maintenance of metabolic pools.

An advance in the technique of permeability measurements, through the use of

filters, has made possible the measurement of the amount of a compound that leaves the cell within a few seconds, and has disclosed errors in the previous interpretations of accessible space measurements.

The direct measurement of cell permeability is carried out essentially as follows: A weighed pellet of cells (with no energy source and no growth occurring) is suspended in a small volume of buffer containing a labeled compound, and centrifuged. The pellet is resuspended in unlabeled buffer and again centrifuged. Assays for the concentration of the labeled compound, carried out on the original supernatant solutions, make it possible to determine the quantity of labeled compound entering or leaving the cell during the 5 to 10 minutes required for suspending and centrifuging. From these results the fraction of the cell volume which is accessible to the compound may be calculated, the concentration within the accessible volume being assumed to be the same as that outside the cell. The meaning of such a volume calculation is discussed below.

The question arises, however, whether some of the compound is held within the cell in a loosely bound form similar to the TCA-soluble pool described earlier in this report. In order to estimate "bound" material a sample of the labeled suspension (just before centrifuging) is diluted into a large volume of unlabeled buffer and instantly filtered on a collodion membrane filter. The time from dilution to completion of the filtering is 5 to 15 seconds. In this time, diffusion equilibrium will be established for molecules free to diffuse from the cells, since the calculated half-time for diffusion equilibrium out of a 1-micron-radius sphere of water is less than a millisecond.

When this procedure is carried out with high concentrations of an amino acid, such as proline or valine, it is found that two-thirds of the amino acid that entered the cell in the first suspension has left the cell before filtering can be completed, that is,

within 15 seconds. When filtering is performed at later times after dilution, it is found that the remaining one-third of the amino acid leaves the cell slowly with a half-time of about 15 minutes. Points taken at close intervals within the first minute show no significant change occurring.

Thus, a clear separation can be made between a fast component and a slow component. The fast component is assumed to be amino acid that is free to diffuse from the cell, and the slow component is presumably bound in a way similar to the metabolic pool material. We are therefore led to define the fraction of the cell volume that would contain the freely diffusible quantity, at the external concentration, as the volume accessible by free diffusion for the particular compound. The values for the accessible volume based on this new definition differ in many cases from previously published values which were not corrected for the "bound," slowly moving material. Earlier measurements, uncorrected for the "bound" amino acids, indicated accessible spaces of about 70 per cent of the cell volume for amino acids. With the new definition the accessible space for free diffusion of amino acids appears to be about 40 per cent of the cell volume.

A schematic diagram of the cell, showing the volumes accessible by free diffusion, can now be constructed as shown in figure 32. It will be noticed that there is a volume inaccessible even to  $D_2O$  which corresponds roughly to the volume of the dried cell material. In addition, there is a volume which is inaccessible by free diffusion to amino acids. The volume accessible by free diffusion for sodium appears to be somewhat larger than that for amino acids or  $PO_4^{3-}$  (about 55 per cent). It should be pointed out that the volume accessible to  $D_2O$  has not been measured with the new rapid technique, and it is therefore conceivable, though unlikely, that part of the 75 per cent volume shown as accessible to  $D_2O$  is not in fact accessible by free diffusion.

The meaning of the concepts *permeable*, *accessible*, and *volume*, as they have been used above, must now be considered in the light of the new evidence presented. Growing cells hold in the pool 1000 times more amino acid than would be contained in the accessible volume at the external concentration. In fact, cells can take up valine into the pool at a rate approaching the maximum set by diffusion toward the cell. Thus, in referring to the permeability of a cell, it must be made clear whether an active or a passive process is being considered. To avoid connotations resulting from previous use or misuse of the word permeability, we prefer the word accessible as in the phrase "accessible by free diffusion."

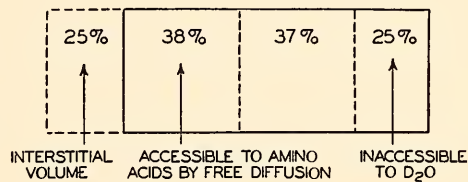


FIG. 32. Schematic diagram of the calculated accessible volumes of the cell.

Careless interpretation of accessible volume measurements, in attempts to judge the availability of a compound for cell metabolism, must be avoided. For instance, the accessible volume by free diffusion for  $SO_4^{2-}$  is only 15 per cent of the cell volume when cell metabolism is at a low ebb in thick suspensions without energy source. Growing cells, however, take up all the sulfur needed for full growth rate even in very low sulfate concentrations. And, strikingly, sulfur-starved cells will take up  $SO_4^{2-}$  at a rate approaching the maximum rate permitted by free diffusion toward the cell. It may *not* be concluded, therefore, that a compound showing a low accessible volume is not readily available for metabolism. On the other hand, a compound showing a large accessible volume may be unavailable to the cells' metabolism. The lack of correlation between per-



meability or accessible volume and the rate of entry of a compound into metabolic processes is not generally realized.

The volume of the cell calculated to be accessible to the given compound by free diffusion does not necessarily correspond to a real physical volume within the cell. The calculation depends on the assumption that the concentration within the volume is the same as that outside the cell, and is thus useful as a simple description of the experimental results. The fact that the accessible volume is independent of concentration supplies partial justification for the assumption.

There are, however, at least four ways in which an accessible volume, 38 per cent of the cell volume, might be interpreted: (1) the concentration over the whole cell is 38 per cent of that outside—in effect a concentration-independent partition coefficient is postulated; (2) the concentration over the whole cell is zero, and the compound is adsorbed on the surface of the cell; (3) the concentration within part of the cell is equal to that outside, but the remainder of the cell is walled off, for example, by a plasma membrane; (4) the concentration in regions scattered throughout the cell is equal to that outside, but a large number of small regions are inaccessible by free diffusion to the compound. These regions may be the individual macromolecules, or larger structures such as the “microsomes” which have been extracted from *E. coli* and photographed by Schachman. It is worth noting that the aggregate volume of these 15-millimicron ribonucleoprotein particles is about 35 per cent of the cell volume.

Finally, these different descriptions may be mixed according to taste. One must, however, choose a description that fits what we know of the real physical organization of the cell and serves as a satisfactory framework for the interpretation of other evidence. The general evidence with regard to metabolic pool formation and the osmotic characteristics of the pool, together with what is known of the physi-

cal organization of the cell, leads us to favor the fourth description.

*Time of protein synthesis.* An upper limit to the time required for the assembly of amino acids into the polypeptide chain of proteins can be established from kinetic studies of amino acid incorporation. If the proteins were synthesized directly from free amino acids in the medium, and if the synthetic process were instantaneous, then the rate of appearance of radioactivity in the protein should rise instantly to a constant value when radioactive amino acids are added to the medium. Any observed delay in the establishment of the final rate would be a measure of the time required in the process of linking the amino acids into the polypeptide chain. From these considerations alone it can be seen that the over-all process, which also includes the entry of the amino acids into the cell, takes less than 20 seconds, as the slope of the time course of incorporation of amino acids into the TCA-insoluble fraction reaches its maximum in less than 20 seconds (fig. 33).

There is, however, a slight initial curvature that implies a finite time for the over-all process. In last year's report it was shown that the amino acids incorporated into protein are taken from the TCA-soluble pool of the cells and not directly from the medium. It is therefore necessary to consider also the time required to build up the radioactivity of the pool material which is the precursor of the protein.

Figure 33 shows the results of an experiment carried out at a low temperature to exaggerate any time requirements for protein synthesis. The total radioactivity incorporated and the TCA-insoluble fraction were measured to find the radioactivity of the TCA-soluble pool by subtraction. The rate of incorporation into protein can then be compared with the specific radioactivity of the amino acid pool. It is estimated that the time required for the transition from TCA-soluble to TCA-insoluble material must be less than



3 seconds to give the observed proportionality.

Other tests were then made to determine whether this transition from TCA soluble to TCA insoluble corresponded to the formation of a completely new protein. It might be possible, for example, that the amino acids were simply linked into small polypeptides, or attached to some previously formed protein. Two cell preparations were made: one in which the cells were exposed to radioactive amino acids for 15 minutes to allow uniform la-

not detect any difference between the newly incorporated amino acids and the other amino acids of the proteins.

In other experiments, cell walls were isolated from two cultures that had incorporated  $C^{14}$  amino acids, one for 15 minutes and the other for 15 seconds. In both preparations the fraction of the radioactivity appearing in the cell walls was the same.

These experiments show the great rapidity of protein synthesis. The transition from TCA soluble to TCA insoluble takes less than 3 seconds. If there are intermediates "in process" there must be less than 1 micromole of amino acids in this form per gram dry cells. Furthermore, in less than 15 seconds the newly incorporated amino acids are indistinguishable from other amino acids and have reached organized structures such as the cell walls.

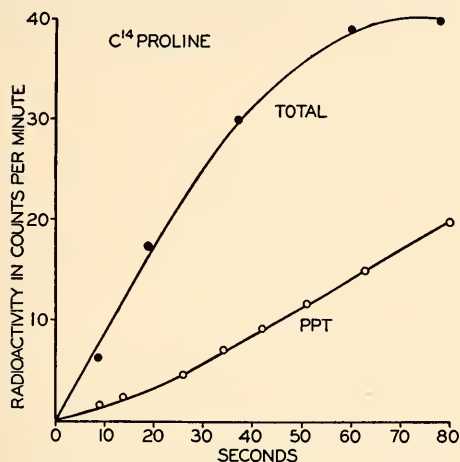


FIG. 33.  $C^{14}$  proline was added to a culture of cells growing at  $18^{\circ}$ . The rate of incorporation into the protein fraction reaches its maximum value in a very short time, showing that there is little partly formed protein in the cell.

beling of the proteins, and another in which the incorporation was allowed to proceed for only 15 seconds.

In these two preparations the proportion of radioactive amino acids in the amino end of the peptide chains was the same. Thus, the newly incorporated amino acids were not simply attached at the ends of a large number of slowly growing peptide chains. The radioactive amino acids could not be broken loose from the protein by oxidation. Also, the rate at which radioactive amino acids were released by acid hydrolysis was the same in both preparations. In short, these chemical tests could

#### *Purine and Pyrimidine Incorporation*

Two years ago (see Year Book No. 53, 1953-1954, pp. 84-91) we reported in considerable detail the results of studies on nucleic acid synthesis in *E. coli*. We were not able by means of the techniques then at our disposal to discover the compounds that are precursors for building the phosphodiester linkages that form the backbone of the nucleic acid molecule. During the past year, application of the membrane-filter technique to the nucleic acid problem has demonstrated the formation and utilization of metabolic pools of precursors, and has shown the role played by phosphorylated intermediates.

The pattern of flow of the nucleic acid building blocks—adenine, guanine, cytosine, and uracil—from the medium to the nucleic acids resembles that for the amino acids during protein synthesis. The bases are taken into the TCA-soluble fraction, concentrated there to form a pool, and subsequently moved on into the TCA-precipitable nucleic acids. Two examples of this kinetic behavior are shown in figure 34.

The incorporation and utilization of the bases require an exogenous supply of energy. This fact is illustrated for  $C^{14}$  adenine in figure 35. It is evident that both the formation of a pool of  $C^{14}$  adenine and the synthesis of radioactive nucleic acid require a supply of energy.

All four bases are found in the pool. In cells growing in a steady state there is more adenine than guanine, and more uracil than cytosine. The adenine or uracil compounds in the pool are at a level of 10 to 20  $\mu\text{mol/gr}$  dry bacteria; the guanine and the cytosine contents are somewhat variable. While the bases are in the pool

they are chemically altered. Adenine is converted to guanine, and cytosine and uracil are interconverted. The base supplied to the cell and the product formed are passed on to the nucleic acid. An example of this precursor-product relation is shown in figure 36. In the early stages the nucleic acid derives much more  $C^{14}$  from pool adenine than from pool guanine. Initially, the proportion contributed by guanine rises very rapidly. After 10 minutes there was no further change because the exogenous supply of adenine was exhausted. When a large supply of  $C^{14}$  adenine is provided, the contribution to the

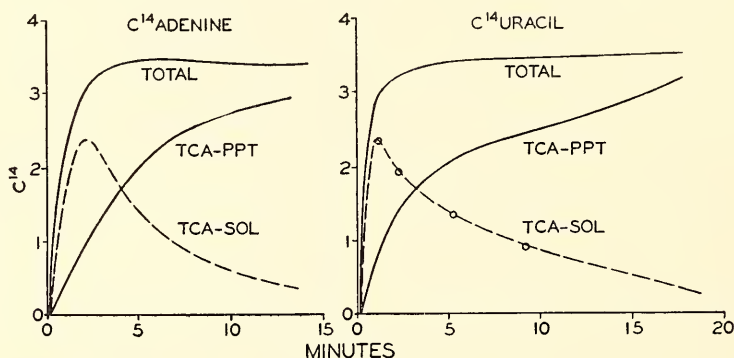


FIG. 34. Uptake and transfer of nucleic acid precursors from the medium to the nucleic acids

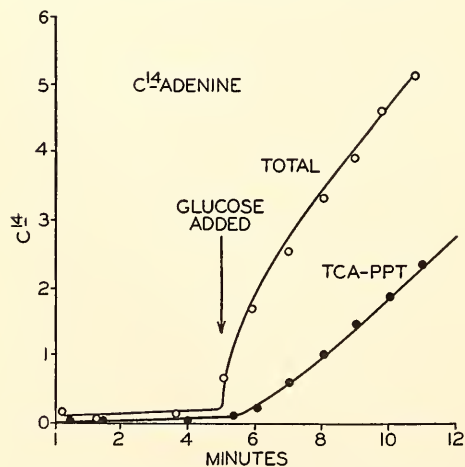


FIG. 35. Energy requirement for adenine utilization.  $C^{14}$  adenine at time zero; glucose added at 5 minutes.

total radioactivity from each of the bases is very nearly equal.

Paper chromatography of alcohol extracts of the radioactive compounds found in the pool, after one of the  $C^{14}$ -labeled bases was supplied to growing bacteria, showed that the bases were combined as phosphorylated derivatives. These derivatives were the precursors to the nucleic acids, as the kinetic data demonstrated. Attempts were made to study the mechanism of  $C^{14}$ -base transfer from the pool to the nucleic acids by means of isotopic competition. No reduction in the uptake of a labeled base by its corresponding nucleoside-5'-mono-, di-, or triphosphate could be shown. This observation raises an interesting question in regard to the permeability of the cell, or the accessibility of spaces in

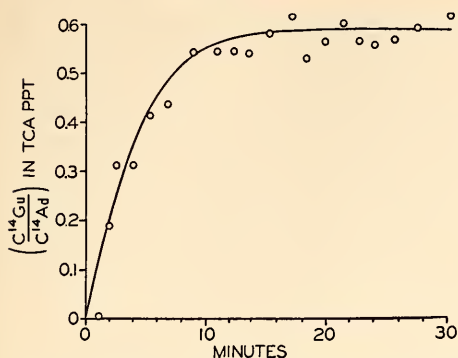


FIG. 36. Rate of appearance of radioactive guanine in the nucleic acid of *E. coli* growing in the presence of  $C^{14}$  adenine.

TABLE 15  
CHEMICAL FRACTIONATION OF *E. coli*

Fraction	P content ( $\mu\text{mol}$ )	Composition	Kinetics
TCA-soluble .....	200	$\text{PO}_4^{3-}$ , phosphorylated bases, etc.	Transient
Alcohol-soluble .....	150	Phospholipids	Stable
Insoluble .....	650	DNA, RNA	Stable

the cell, to the phosphates. It was our hope, however, to uncover facts about the chemical mechanisms for assembling nucleic acid from its precursors. Consequently, we focused upon the kinetic relations during the transfer of radioactive phosphorus from the medium, through the pool, and into the nucleic acids. These studies are described in detail in the following section.

### Phosphorus Incorporation

An extensive study of the flow of phosphorus in the metabolism of *E. coli* has been carried out during the past year. Phosphorus is a constituent of the nucleic acids, and phosphorus compounds such as adenosine triphosphate (ATP) have long been thought to be involved in the energy transfer mechanisms. Accordingly, it was considered possible that a detailed knowledge of the kinetics of phosphorus transfer might provide some clues either to the mechanisms of nucleic acid synthesis or to the way in which energy is supplied for protein synthesis.

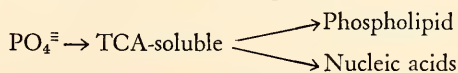
*E. coli* contains approximately 1000 micromoles of phosphorus per gram dry cells. In the growing cells the phosphorus is in three classes of compounds which are readily separated by simple extraction procedures, as shown in table 15.

*Kinetics of the fractions.* As simple extraction with TCA removes the transient intermediate compounds, the filter technique described above can be used to explore many of the general features of phosphorus metabolism.

When  $\text{P}^{32}\text{O}_4^{3-}$  is added to a growing culture of *E. coli*, and samples are taken to measure both the total incorporated and

the TCA-insoluble fraction, the curve shown in figure 37 is obtained. (In all these experiments using  $\text{P}^{32}$  tracers, the usual phosphate buffer is replaced by tris-hydroxymethylaminomethane, "TRIS.")

The slow rise of the TCA-insoluble fraction suggests that it is derived from the soluble fraction according to the model:



According to this model, the radioactivity incorporated into the soluble fraction ( $Y$ ) per unit quantity of cells ( $Q$ ) should be given by:

$$\frac{Y}{Q} = \frac{Y_0}{Q_0} (1 - e^{-bt})$$

where  $Y_0/Q_0$  is the steady-state value (200  $\mu\text{mol}/\text{gr}$ ) and  $b$  is a constant related to the rate of phosphorus incorporation. The observed curve is of this form, and the constant  $b$  can be determined.

Furthermore, when the cells are growing exponentially,  $Q = Q_0 e^{at}$ , where the

constant  $a$  is given by the growth rate. Finally, the model predicts that the radioactivity incorporated into the insoluble fraction ( $Z$ ) should be given by:

$$\frac{Z}{Q} = \frac{Z_0}{Q_0} \frac{1}{b-a} [b(1-e^{-at}) - a(1-e^{-bt})]$$

Figure 37 shows that this is the case, and the model gives a good approximation

in addition to the 19  $\mu\text{mol/gr/100 sec}$  incorporated. This exchange loss of phosphorus is somewhat variable, and sometimes runs as high as 20  $\mu\text{mol/gr/100 sec}$ .

The stability of the TCA-insoluble fraction and the exchange loss from the soluble fraction can be shown in a more direct way by adding a large quantity of  $\text{P}^{31}\text{O}_4^{3-}$ , which dilutes the  $\text{P}^{32}\text{O}_4^{3-}$  to such an extent

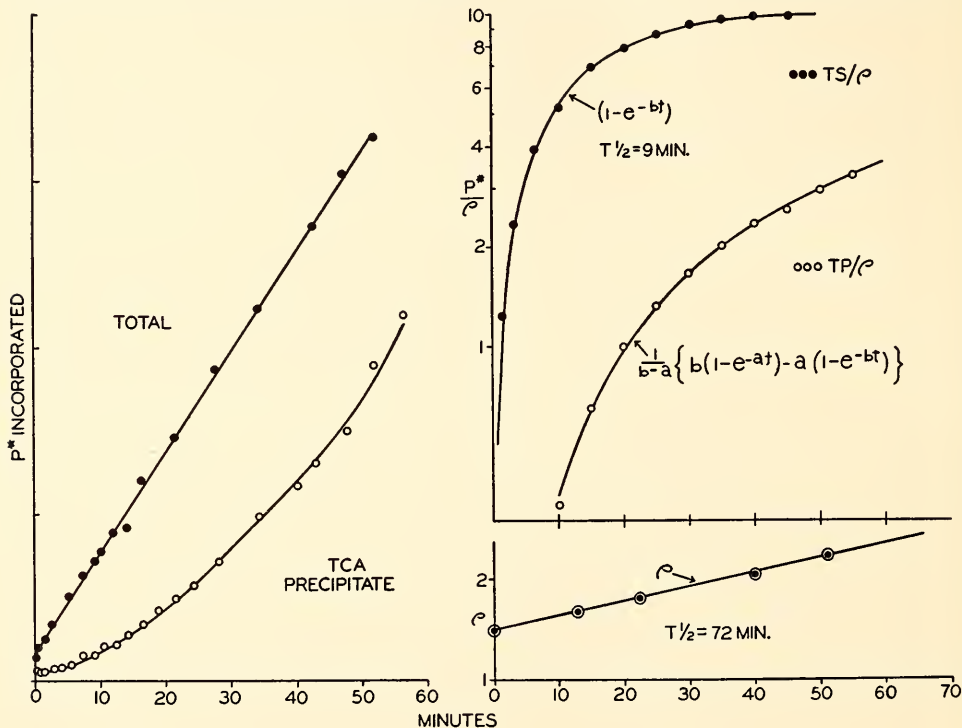


FIG. 37. The incorporation of  $\text{P}^{32}\text{O}_4^{3-}$  by growing *E. coli*. The curves on the left are the experimental data. The same data are plotted in a different way for comparison with the curves calculated from the equations given in the text.

of the kinetics of phosphorus incorporation.

If the cells are growing in a steady state with a generation time of 60 minutes, they require a rate of incorporation of 1.9 per cent/100 sec or 19  $\mu\text{mol/gr/100 sec}$  to provide the phosphorus required for growth. The constant  $b$  evaluated above showed that the actual rate of incorporation is somewhat higher (25  $\mu\text{mol}$ ), which implies an exchange of 6  $\mu\text{mol/gr/100 sec}$

that its incorporation is reduced to a very low rate. As is shown in figure 38, after the  $\text{P}^{31}\text{O}_4^{3-}$  is added the  $\text{P}^{32}$  content of the TCA-soluble fraction decreases exponentially, roughly one-half being lost to the medium and the other half being converted to nucleic acid and phospholipids.

It should be stressed that the equations above give only a good approximation to the kinetics of phosphorus metabolism. As will be shown below, the composition of



the TCA-soluble fraction is very complex, and only a portion contributes phosphorus to nucleic acid. These different compounds of the soluble fraction equilibrate among themselves rapidly so that extreme precision is necessary to show any deviations from the equations based on the simple model above. In addition, a part of the phosphorus of the soluble fraction is contained in kinetically stable molecules, but this fraction is so small that it does not introduce any deviation observable in an experiment of this type.

*Conditions for phosphorus uptake.* When glucose is omitted from the medium, no

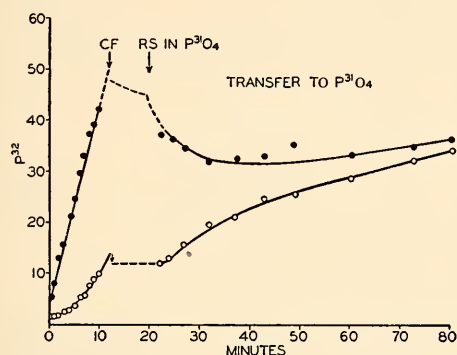


FIG. 38. Loss of  $P^{32}$  from the TCA-soluble fraction. Cells were grown in the presence of  $P^{32}O_4^{3-}$ , then transferred to a  $P^{31}O_4^{3-}$  medium. The radioactivity of the TCA-soluble fraction is partly lost to the medium and partly converted to TCA-insoluble compounds.

phosphorus is incorporated. In a similar way, when the supply of glucose is exhausted the incorporation stops. These conditions are shown in figure 39. Neither lactate nor uridine added singly will replace glucose as an energy source, but a combination of the two will give some growth and phosphorus incorporation. This finding is in accord with other experiments which show that uridine accelerates adaptation to new carbon sources such as lactate, pyruvate, and acetate, perhaps by providing ribose in a usable form.

When nitrogen is omitted from the medium, or after the supply of nitrogen is

exhausted, there is no uptake of phosphorus. This is quite surprising, since the consumption of glucose continues at about one-half rate and potassium (which like phosphorus is involved in glycolysis) is exchanged. At first sight it might appear that phosphorus is needed only when nucleic acid is being synthesized. This is not the case in an adenine-requiring mutant of *Salmonella* (obtained from Dr. M. Demerec), which is closely related to *E. coli*. When nucleic acid synthesis is blocked by lack of nitrogen there is no uptake of phosphorus. In contrast, when nucleic acid synthesis is blocked by lack of adenine, phosphorus does enter the TCA-soluble fraction. It appears that the synthesis of some nitrogen-containing compound is necessary for the entry of phosphorus into the pool.

The addition of chloramphenicol at a concentration of 10  $\gamma$ /ml, which reduces protein synthesis to one-twentieth its normal rate, has no immediate effect on phosphorus incorporation. Nor can any change in the phosphorus uptake be observed when amino acids are added. Evidently any connection between protein synthesis and phosphorus metabolism is too subtle to be easily observed by measurements of the kinetics of phosphorus transfer between the major fractions.

*Competition.* A number of compounds have been tested as competitors with  $PO_4^{3-}$ . Most of them, including adenosine triphosphate (ATP), glucose-1-phosphate,  $\alpha$ -glycerol phosphate, etc., caused no reduction in the uptake of  $P^{32}O_4^{3-}$ . When glucose-6-phosphate, phosphoenol pyruvate, or fructose-6-phosphate (F6P) was added at concentrations equal to the concentration of phosphate (0.25  $\mu$ mol/ml), the uptake of  $PO_4^{3-}$  was markedly affected (fig. 40).

Two features of this curve require comment. The reduction does not occur immediately but only after a 5-minute lag, in marked contrast to other known competitive effects. The lag indicates that these compounds act indirectly, perhaps by raising the level of phosphate com-

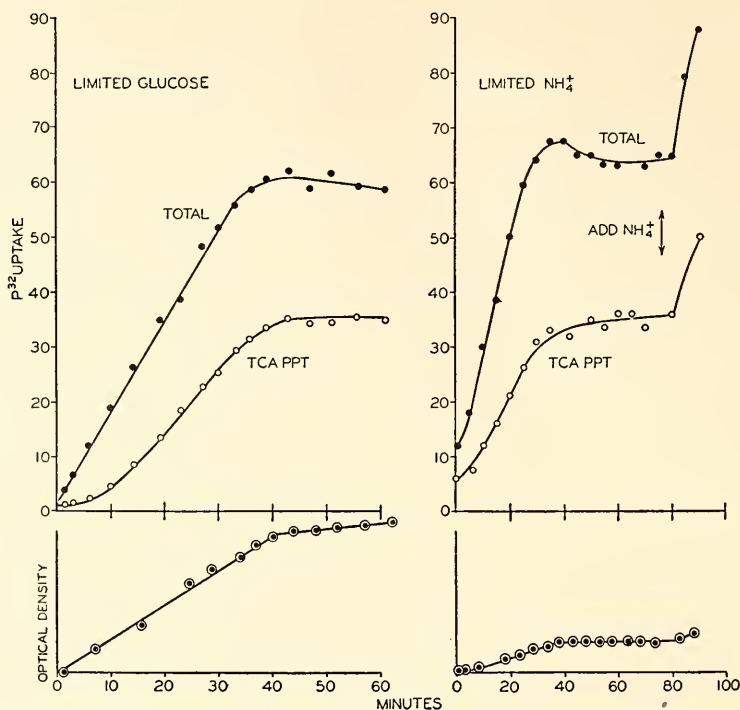


FIG. 39. The incorporation of phosphorus stops when the medium becomes depleted of glucose or  $NH_4^+$ .

pounds within the cell. Secondly, the uptake of  $PO_4^{3-}$  is resumed at one-half the previous rate after a short period. Evidently the F6P is used up at a rate corresponding to the rate at which the cell uses glucose ( $400 \mu\text{mol/gr}/100 \text{ sec}$ ). Most of its phosphorus returns to the medium as  $PO_4^{3-}$  and dilutes the  $P^{32}O_4^{3-}$ . The rate of

dephosphorylation is 10 times higher than the usual rate of phosphorus intake. This situation is surprising, because earlier experiments have shown that F6P supplies little carbon to the cell when glucose is also present. The findings are difficult to reconcile with the usual concepts of glycolysis.

*Osmotic shock.* The phosphorus compounds of the TCA-soluble pool exhibit a sensitivity to osmotic shock which is in some ways similar to, and in some ways different from, the osmotic sensitivity of the amino acid pool. To date, the experiments done with the phosphorus compounds have been carried out with cells grown in TRIS medium whereas the amino acid tests used C medium. No great difference would be expected between the two media, but the results are not strictly comparable until the tests have been repeated in the same medium.

Table 16 shows the results of a prelimi-

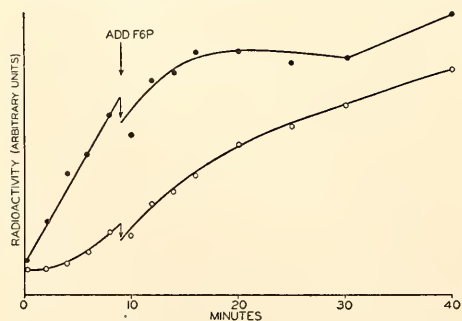


FIG. 40. The incorporation of phosphorus from  $P^{32}O_4^{3-}$  stops 5 minutes after fructose-6-phosphate is added to the medium.

nary survey obtained by washing the cells on the filter with various fluids. As a large number of phosphorus compounds are found in the soluble pool, chromatograms were made of the material remaining after washing with several of the fluids. Phosphate appears to be the most readily extracted compound.

Phosphorus metabolism has another and different sensitivity to dilution. If a growing culture is diluted with water, the up-

TABLE 16

REMOVAL OF TCA-SOLUBLE PHOSPHORUS BY  
WASHING

Washing fluid	Osmotic strength	Per cent removal
TRIS .....	0.31	0
	.15	35
	.04	55
	.01	75
Water .....	0	85
Glucose .....	.83	15
	.37	0
	.18	60
	.09	75
	.04	85
Sodium chloride .....	.31	0
Sodium acetate .....	.31	0
Sucrose .....	.31	0
Urea .....	.31	30
Glycerol .....	.31	80
Ethanol .....	0.31	85

Cells were grown with a limited glucose supply to give a constant pool of TCA-soluble phosphorus. They were removed from the medium by filtering, and washed while still on the filter with 2 ml of the listed fluids.

take of phosphorus into the cell as a whole stops abruptly for a period of several minutes and then is resumed at the original rate. Dilutions as low as 20 per cent, which remove very little phosphorus from the cell, will produce this effect. The treatment has no effect on the transfer of phosphate to the insoluble fraction, and is presumably connected with the mechanism involved in the entry of external phosphate.

The differences between the removal of phosphate and the removal of amino acids

may be due in part to differences in their localization within the cell. It is also possible that different-sized compounds might require different degrees of osmotic shock to be released from the same structure. In any event, the differences do demonstrate that the various components of the TCA-soluble pool are not all held within a common membrane that simply breaks on osmotic shock.

*Composition of the soluble fraction.* For further insight into the metabolism of

TABLE 17

STEADY-STATE DISTRIBUTION OF PHOSPHORUS  
COMPOUNDS IN THE TCA-SOLUBLE POOL

Compound	Micromoles per gram dry weight
PO <sub>4</sub> <sup>=</sup> .....	80
ATP .....	20
ADP .....	12
GTP .....	7
GDP .....	7
UTP .....	15
UDP .....	20
CTP .....	5
CDP .....	5
10 unidentified spots .....	29
Total .....	200

phosphorus it is necessary to observe the individual components of the TCA-soluble fraction. For this purpose the cells are filtered and washed; the filter is promptly placed in a beaker containing 75 per cent ethanol. The TCA-soluble fraction and the phospholipids are extracted, leaving the nucleic acid still on the filter. The alcohol extract is then reduced in volume and applied to a sheet of Whatman No. 1 paper for two-dimensional chromatography.

Figure 41 shows a typical radioautograph of a chromatogram and indicates the spots that have been identified. The steady-state distribution is given in table 17. Most of these compounds are transient, and their quantity is affected by the differ-

ence between supply and demand. Hence, it is not surprising that the composition is somewhat variable, depending on the growth conditions. The guanine and cytosine compounds that are present in low concentration are subject to the greatest variations.

That some of these compounds are kinetically stable can be shown by growing cells in  $P^{32}O_4^{=}$  until a steady state is obtained and then replacing the  $P^{32}O_4^{=}$  by  $P^{31}O_4^{=}$ . The transient compounds then decrease in radioactivity while the end products lose little of their radioactivity. This situation is shown in figure 42, where three minor components of the steady state are the most prominent. These compounds have not been identified, but they may well be coenzymes. The phospholipid also is a kinetically stable end product. The steady-state pattern of the pool has a remarkable degree of constancy. Cells that lack glucose might be expected to be deficient in the high-energy triphosphate compounds. They are not. Chromatograms of the soluble phosphorus compounds taken from cells which were incubated for 1 hour in media lacking either glucose,  $NH_4^+$ , or  $PO_4^{=}$  showed no significant change from the steady-state distribution. Even an adenine-requiring mutant showed no lack of adenine compounds after its growth was stopped by lack of adenine. This stability of the phosphorus system is in marked contrast to the instability of glutathione, which is completely used up when the cells lack sulfur.

*Kinetics of the individual compounds.* Some information about the source of nucleic acid phosphorus was obtained from studies of the kinetics of the individual phosphorus compounds of the soluble fraction. Cells were grown at 18° in a very low phosphorus medium (0.01  $\mu\text{mol/ml}$ ).  $P^{32}O_4^{=}$  was added to the culture, and after 1 minute's exposure all further uptake of  $P^{32}$  was stopped by diluting the culture medium with a large excess of  $P^{31}O_4^{=}$  (50  $\mu\text{mol/ml}$ ). Thus a pulse of radioactivity was taken into the cells, and its prog-

ress could be traced (fig. 44). Samples were taken at intervals for chromatography and to measure the incorporation into nucleic acid. In a chromatogram of the earliest sample, taken after 12 seconds, only the  $PO_4^{=}$  and the triphosphates of the nucleic acid bases show appreciable radioactivity (fig. 43). These compounds also showed a rapid decrease in radioactivity after the incorporation had stopped, then a secondary rise and decline (fig. 45). Radioactivity appeared more slowly in the diphosphates and reached a maximum later.

It seemed likely that the outermost phosphate of the triphosphate was responsible for the rapid rise and decrease whereas the secondary rise of the triphosphates was due to the appearance of radioactivity in the inner two phosphorus atoms. This hypothesis was tested and confirmed by degrading the ATP and uridine diphosphate (UDP) and measuring the radioactivity of each phosphorus atom separately. Figure 46 shows the radioactivity of the individual phosphorus atoms of ATP. The radioactivity appears first in the outer phosphorus, more slowly in the second phosphorus, and only after a considerable delay in the inner phosphorus.

These results give considerable insight into the circulating flows of phosphorus compounds.  $PO_4^{=}$  and the four triphosphate compounds rise very quickly together, implying a rapid circulation that keeps them in equilibrium. The flow of glucose to pyruvate is 200 to 300  $\mu\text{mol/gr/100 sec}$ , and each mole of glucose should transfer 2 moles of phosphate to the triphosphates. Accordingly, the rapid equilibration of  $PO_4^{=}$  and the external phosphorus of the triphosphate is not unexpected. This mechanism would not transfer any phosphorus to the interior positions.

The slow rise of the innermost phosphorus atom can also be explained. As the phosphorylated bases are incorporated into nucleic acid, newly formed ribosides must be phosphorylated. These reactions would



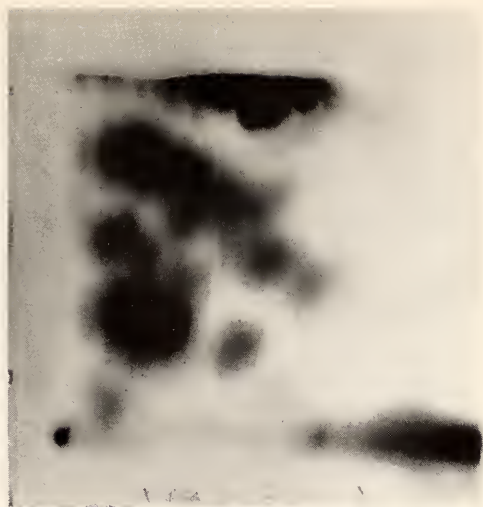
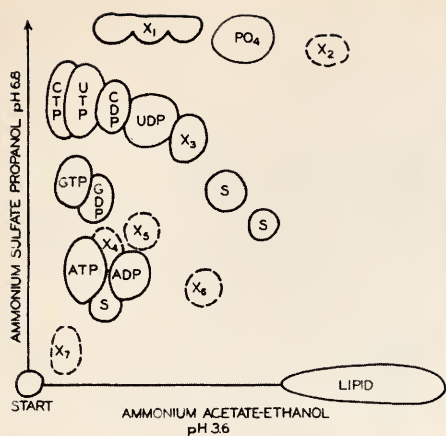


FIG. 41. Radioactive compounds which appear after paper chromatography of alcohol extracts of cells grown in the presence of  $P^{32}O_4^{3-}$ . The diagram shows the location of identified compounds. *S* indicates that the compound is a stable end product.



FIG. 42. Only a few compounds of the alcohol extract are kinetically stable and retain their radioactivity after an hour's growth in a medium containing  $P^{31}O_4^{3-}$ .



FIG. 43. This series of chromatograms shows how the radioactivity of a pulse of  $\text{P}^{32}\text{O}_4^{3-}$  appears first in the triphosphates and  $\text{PO}_4^{3-}$  and is transferred later to other compounds. Times: 12 seconds, 55 seconds, 3 minutes, and 14 minutes.

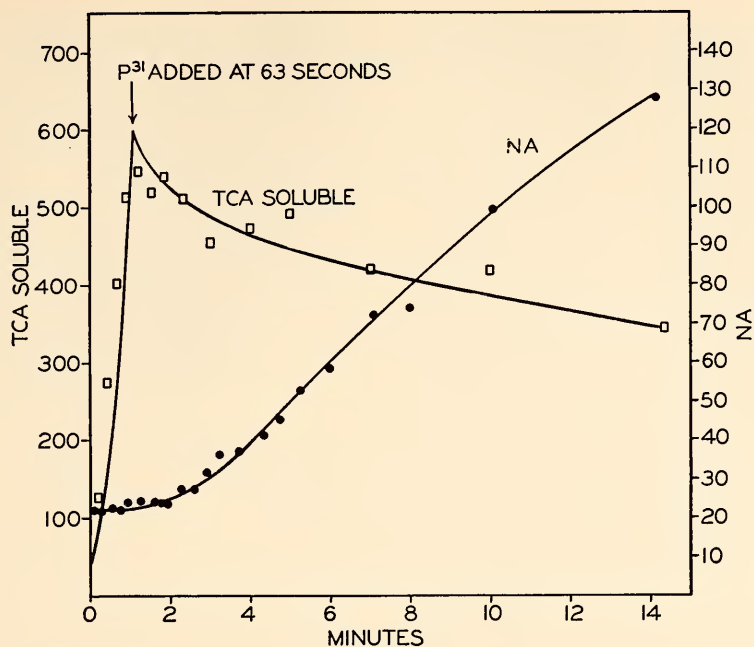


FIG. 44. Cells were exposed to high specific radioactivity  $P^{32}O_4^{3-}$ , and after 63 seconds  $P^{31}O_4^{3-}$  was added; the radioactivity rapidly enters the TCA-soluble fraction and appears later in the nucleic acid fraction.

transfer phosphorus from the outer position to the inner positions at a relatively slow rate because the new ribosides are formed at roughly  $15 \mu\text{mol/gr}/100 \text{ sec}$ . Several minutes would be required to

bring the interior phosphorus to equilibrium by these reactions—roughly corresponding to the time interval observed.

*Source of nucleic acid phosphorus.* When the cells are growing in a steady state and the addition of the  $P^{32}$  causes no disturbance to the system, it can be assumed that the rate of incorporation of radioactivity into the nucleic acid will be proportional to the specific radioactivity of the nucleic acid precursors. Figure 43 shows that there is a very definite lag period in the incorporation of  $P^{32}$  into nucleic acid. This implies that a corresponding time elapses before the specific radioactivity of the nucleic acid precursors rises. Referring to figure 46, it can be seen that the nucleic acid phosphorus must be derived from the innermost phosphorus atom of the nucleotides, as the specific radioactivity of the other phosphorus atoms rises too rapidly. Further experiments are needed to determine whether one or all of the nucleotides contribute

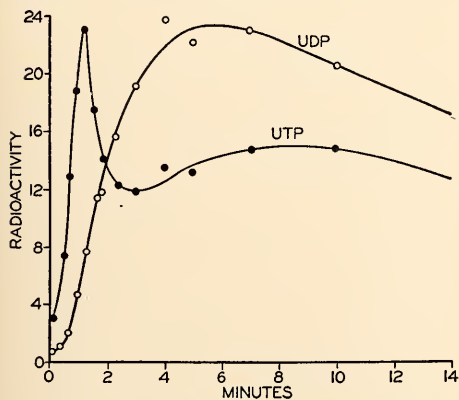


FIG. 45. The radioactivity of uridine-triphosphate and uridine-diphosphate was measured on the chromatograms. The other di- and triphosphate compounds showed similar kinetic behavior.

phosphorus to nucleic acid. This finding is consistent with the polymerization of diphosphate nucleotides observed in cell-free extracts by Grunberg-Manago and Ochoa.

*Phosphorus metabolism and protein synthesis.* No evidence for a direct relation between phosphorus metabolism and protein synthesis has been found. Chloramphenicol, which stops protein synthesis, has no effect on the incorporation of phosphorus. Cells in which the incorporation of amino acids is blocked by a lack of glu-

the growth of bacteria, reduces the rate of cell division in certain carcinomas, and aids in the treatment of myelocytic leukemias. Several investigators have suggested that the drug might exert its effect upon growth and cell division by interfering with nucleic acid synthesis. Although the mechanism of the action of the drug has not been established, it has been suggested that 6-MP may specifically suppress or alter nucleic acid synthesis. Since such an agent might provide the biologist a means for studying the relation between

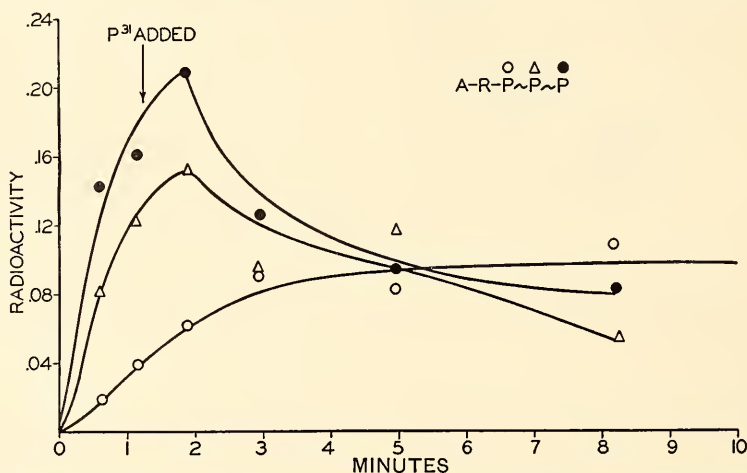
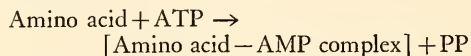


FIG. 46. Adenosine triphosphate was degraded to allow separate measurements of the radioactivity of the three phosphorus atoms. The outermost phosphorus atom is the first to pick up the radioactivity.

cose still contain plenty of nucleotide triphosphate which might be expected to supply the energy required for incorporation. No pyrophosphate is found in the cells. In spite of a prolonged search we have found no positive indication of the reaction:



which may be involved in the incorporation of amino acids.

#### *The Effects of 6-Mercaptopurine on Biosynthesis in E. coli*

6-Mercaptopurine (6-MP) is a chemical analogue of the purine adenine. It inhibits

nucleic acid synthesis and growth, a systematic study of the effects of 6-MP on biosynthesis in *E. coli* was undertaken. These studies were carried out in cooperation with Dr. H. George Mandel, Department of Pharmacology, George Washington University Medical School.

The addition of 6-MP (at 10  $\mu\text{gr}/\text{ml}$ ) to exponentially growing cultures decreased the growth rate of *E. coli*. If smaller amounts (e.g., 1  $\mu\text{gr}/\text{ml}$ ) of 6-MP were used, growth inhibition was observed, but upon prolonged incubation the growth rate of the bacteria returned to normal. Reversal of growth inhibition, even at the higher drug level, could be achieved by



adding the normal purine adenine to the cultures. When the bacteria were exposed to the drug for 10, 30, or 60 minutes and then treated with adenine, normal growth resumed only after considerable delay. The length of the lag period between addition of adenine to 6-MP inhibited cultures and onset of the normal growth rate was a function of the time of exposure of the cells to the drug. These observations suggested that 6-MP exerted at least two effects upon the cell: it interfered with cell activities required for optimal growth, and it interfered with the synthesis of cell components from purines. It was clear, however, that in these experiments the drug did not cause permanent cell damage.

Our previous work on biosynthesis in *E. coli* has shown that  $C^{14}$  glucose labels all the bacterial carbon compounds;  $P^{32}O_4^{=}$  labels chiefly the lipids and nucleic acids;  $S^{35}O_4^{=}$  labels principally the proteins;  $C^{14}$  acetate labels both proteins and lipids, and radioactive formate, purines, and pyrimidines label the nucleic acids. Experiments with this array of radioactive materials were therefore carried out in an attempt to discover which areas of bacterial biosynthesis might be specifically influenced by 6-MP.

The results with  $C^{14}$ -labeled formate, uracil, or glucose showed that 6-MP (10  $\mu$ gr/ml) caused a marked decrease in (a) the rate of uptake of radioactivity, and (b) the amount taken up per unit weight of the bacteria grown during the experiment. When nonradioactive adenine was added to 6-MP-inhibited bacteria which were utilizing  $C^{14}$  uracil, the rate of uptake of radioactivity increased until the amount of  $C^{14}$  uracil utilized per unit of new growth was the same as that in the control culture. These results were consistent with the idea that 6-MP suppresses nucleic acid synthesis. As a check, the nucleic acid content of 6-MP-inhibited bacteria was measured by spectrophotometry and by means of radioactivity derived from  $C^{14}$  fructose and  $P^{32}O_4^{=}$ . It was found that the nucleic acid content of 6-MP-treated

bacteria was from one-half to three-quarters that of the normal cells. Spectrophotometric examination of concentrated solutions of nucleic acid from drug-treated bacteria failed to reveal the presence of 6-MP, which has a characteristic absorption in the range 300 to 340 m $\mu$ , and the carbon-to-phosphorus ratio was the same as that in the nucleic acid from untreated cells.

The result with  $S^{35}O_4^{=}$  and  $C^{14}$  glucose showed that the rate of protein synthesis in cultures of 6-MP-treated bacteria was less than that of normal cells. In addition, the rate of formation of the inducible enzyme  $\beta$ -galactosidase was decreased by drug treatment. These effects, however, were in exact accord with the decrease in the over-all growth rate. Thus, the protein content of the 6-MP-treated bacteria remained the same as that of the untreated organisms.

The results with  $C^{14}$  glucose showed little effect of 6-MP upon the lipid or protein content of *E. coli*. The drug caused a marked suppression, however, in the utilization of 1- $C^{14}$ -acetate, which also labels both lipid and protein. It appeared, therefore, that another effect of 6-MP was to alter the flow of carbon from acetate into the lipids and proteins.

The rate of growth, the nucleic acid content, and the flow of acetate carbon in *E. coli* were decreased by 6-MP treatment. These effects differed, however, in sensitivity to the drug, as separate experiments using several drug levels showed. In order of sensitivity to 6-MP the metabolic properties examined fall into the sequence: growth, acetate utilization, formate utilization and nucleic acid content, protein and lipid content. Thus, the bacterial growth rate can be reduced by 6-MP without concomitant reduction in the nucleic acid content. Therefore, factors other than the nucleic acid content must be determining the growth rate. These experiments, however, have not revealed the elements which are affected by 6-MP with a resultant decreased rate of growth. Nevertheless, they specify the nature of a site

of action of the drug. Both the function and the synthesis of the drug-sensitive system can be affected, as the growth experiments show. The function of the system involves the mechanisms that regulate the flow of carbon from glucose and acetate into protein and lipid. The system functions in the early stages of acetate utilization, since the largely independent pathways into lipid and protein are both involved. The synthesis of the system involves purine precursors, for purine compounds can reverse the effects of the drug.

By analogy with other antimetabolite actions it may be expected that 6-MP affects a limited group of compounds. Coenzyme-A, adenosine-triphosphate, and diphosphopyridine nucleotide meet the specifications, inasmuch as they take part in the early stages of acetate utilization and also contain purine residues in their structures. Coenzyme-A, in addition, contains a sulfhydryl group which could conceivably be competed with or inactivated by coupling with 6-MP. It is suggested, therefore, that 6-MP exerts its effects on *E. coli* by interfering with the function and synthesis of purine-containing coenzymes. Recent reports, which demonstrate the reversal by coenzyme-A of 6-MP-induced inhibition of mitosis in sarcoma 180 and the inhibition by 6-MP of diphosphopyridine nucleotide synthesis and breakdown, lend support to this hypothesis.

#### *Fractionation of E. coli Nucleic Acids*

Current theories of protein synthesis involve ribonucleic acid (RNA) as the structure that determines the sequence of amino acids in proteins. Implicit in this faith is the assumption that for every specific protein there must be a corresponding specific nucleic acid. This idea has already found expression in the one gene-one enzyme hypothesis of Bonner, wherein a specific gene, which can be approximately equated to a DNA molecule, controls the synthesis of a specific enzyme. Thus one could envision genes, specific DNA molecules, operating through specific

RNA molecules (templates), causing the synthesis of specific proteins.

One good way to study specific protein synthesis is to examine the formation of enzymes. One class of enzyme, found mainly in microorganisms, is produced only under the influence of specific molecules present in the environment. Such enzymes are termed adaptive or inducible. One of the best known is the  $\beta$ -galactosidase of *E. coli*, which is synthesized by the cells only when a suitable molecule (inducer) is present in the environment.

When the two above ideas are combined it appears reasonable to suggest that when an inducible enzyme is produced by the cells there will be concomitant synthesis of a specific RNA template. It has been shown by many workers that the synthesis of inducible enzymes will proceed only when RNA is being synthesized at the same time. An investigation was, therefore, made of nucleic acid synthesis in *E. coli* during  $\beta$ -galactosidase formation.

In order to search for a specific RNA molecule it is necessary to separate the different RNA molecules. Fortunately, a chromatographic method, using columns of chemically modified cellulose (ECTEOLA), has been developed by Sober and Peterson, of the National Institutes of Health, and utilized by Bendich and his associates for the fractionation of DNA.

To extract and purify nucleic acid from the cells of *E. coli* a standard procedure was followed, involving precipitation of nucleic acid by the cationic detergent (cetyltrimethylammonium bromide [CTAB]). The nucleic acid was examined after adaptation had occurred, and no difference was observed from a control preparation that had not formed any  $\beta$ -galactosidase.

Our more recent experiments found that the procedure of extraction by CTAB results in the recovery of a constant but nonrepresentative fraction of the cellular RNA. It is possible to obtain higher yields by precipitating the nucleic acid with alcohol instead of CTAB. Figure 47 shows

the elution diagram of nucleic acid obtained by these two procedures. Work is now under way to determine whether any different nucleic acid synthesis takes place during adaptation in the fraction precipitated by alcohol which was previously discarded.

### Components of *E. coli* Cells

It was shown in last year's annual report that cells of *E. coli* can be broken and

Centrifugation of the material from the pressure cell at  $20,000 \times g$  for 15 minutes at  $5^\circ \text{C}$  gives a supernatant free from microscopically visible material. This supernatant will bring about the formation of  $\text{P}^{32}$ -labeled ATP from  $\text{P}^{32}\text{O}_4^=$  and either ATP, ADP, or AMP. If the supernatant is dialyzed for 30 minutes at  $5^\circ \text{C}$  before incubation with labeled  $\text{PO}_4^=$ ,  $\text{P}^{32}$ -labeled ATP generation from either AMP or ADP is dependent upon a supply of

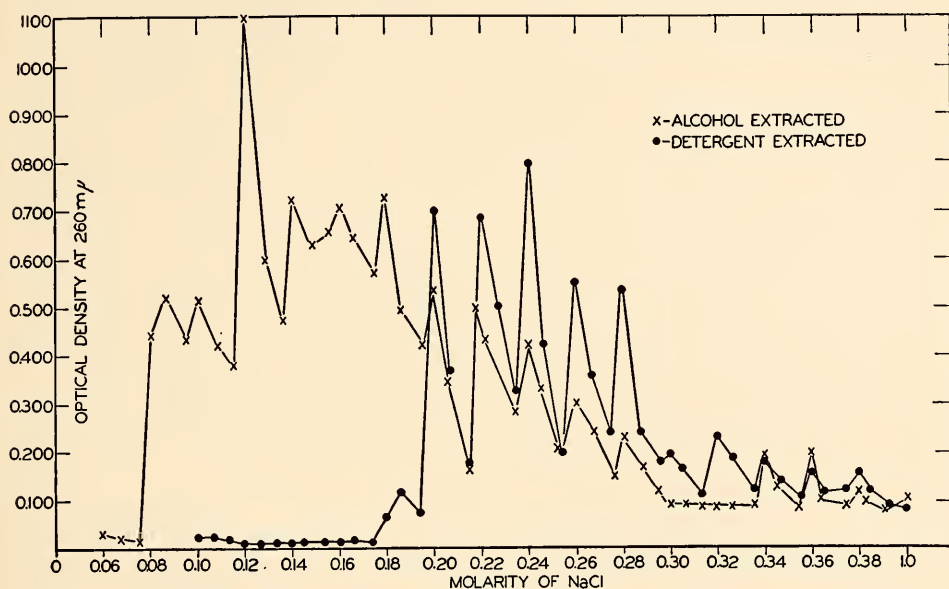


Fig. 47. Nucleic acid elution patterns from ECTEOLA columns

the cellular contents released very effectively by means of the pressure cell designed by C. S. French. When the resulting fluid is examined microscopically, few whole cells, many cell walls, and many particles can be observed. The particles are dense under phase contrast, and are 200 to 300 millimicrons in diameter. These bodies are, in all probability, artifacts of preparation, being composed of collapsed, rolled-up cell walls, with a variable amount of accreted cytoplasmic debris. A clean preparation of cell walls left standing over night changes from a collection of flat, translucent ghosts into these spherical bodies.

energy, such as succinate or pyruvate, and upon the presence of coenzyme-1 and coenzyme-A. It is further enhanced by the addition of cysteine and magnesium ions. This system will oxidize acetate, citrate,  $\alpha$ -ketoglutarate, pyruvate, and succinate. Oxidation of succinate is prevented by malonate.  $\text{C}^{14}$ -labeled glutamic acid is partly converted to  $\text{C}^{14}$ -labeled aspartic acid. It seems, therefore, that this extract contains the Krebs cycle enzymes. The work of Schachman *et al.* has shown that extracts of *E. coli* contain particles 15 millimicrons in diameter, and, if the Krebs cycle enzymes are particulate, they could be on these particles. This is a different situa-



tion from that in mammals, where the Krebs cycle enzymes are on large particles, mitochondria, which are as much as 1 micron in diameter. By analogy with mammalian systems, these particles of *E. coli* correspond to the ribonucleoprotein of the microsome fraction, and hence would be expected to incorporate amino acids. To date, the *E. coli* extract has not done this.

### Conclusions

The experimental facts outlined above are not sufficient to be assembled into any definite picture of protein or nucleic acid synthesis. Yet it is worth the effort to assemble them into some sort of hazy scheme, partly for the record, to show how near or how far from the final answer we were in 1956, and partly to provide a target for the next year's experiments.

It seems quite certain that exogenous materials are concentrated by the cells before their use for synthesis. Some of the materials are chemically altered and stored in the altered form—thus adenine is taken up by the cells and rapidly converted to ATP. Glucose is rapidly converted to a wide variety of compounds found in the TCA-soluble pool before its carbon appears in the macromolecules. Other materials, amino acids especially, seem to remain unchanged while they are in the soluble fraction. Carbon supplied to the cell as proline is in part converted to glutamic acid and arginine, but most remains as proline and is incorporated into the protein as proline. No activated forms of amino acids have been detected.

Even though some alterations do occur, there is no evidence of any partly formed fragments of protein or nucleic acid. Instead, quite low limits can be placed on the possible quantities of peptides or small polynucleotides, as any sizable pool of these materials could be detected either by chromatography or by a kinetic delay in the incorporation of tracer materials.

Nor is there any unusual distribution of newly incorporated amino acids in what

is presumably newly formed protein. Rather, amino acids seem to be distributed at random throughout the peptide chains. Accordingly, we are forced to the conclusion that synthesis of the proteins takes place with great rapidity, and that, in a matter of a few seconds at most, the small building blocks find their proper order and are linked together. This course of events is in accord with the commonly accepted idea that order arises from order, and that pre-existing molecules act as the templates for newly forming ones.

The rapidity of the process is perhaps surprising. There are roughly six times as many amino acids in the proteins of *E. coli* as there are nucleotides in the RNA, and during a generation the quantity of each doubles. If we assume that RNA acts as the template for protein synthesis, and that two nucleotides are required to determine the proper amino acid for each location, then it would be only necessary for each template to act twelve times during one generation. At 18° C, where the generation time is 3½ hours, 16 minutes could be allowed for the amino acids to find their proper place on the template. Instead, the actual time taken was less than 3 seconds. It is possible that only a small part of the nucleic acid is active, or that protein synthesis takes place during only a small fraction of the generation time. It is also possible that the amino acids move on and off the template by exchange until the instant that the peptide chain is formed. It seems much more likely, however, that whenever a template is exposed it is rapidly covered with amino acids, which then link to form a completely new polypeptide. The time-consuming process could then be the stripping of the protein from the template.

The newly formed nucleic acids have not yet been examined, but the transition from small TCA-soluble nucleotides to TCA-insoluble nucleic acid seems to be equally rapid.

This type of mechanism implies that there is little chance of finding any inter-



mediates and little hope of gaining further clues about the mechanism from an examination of the end products. The behavior of the small molecules before their assembly into macromolecules seems the most likely source of information on the synthetic process. Accordingly, we must consider the properties of the pool materials.

Exogenous amino acids present in the medium are concentrated by growing cells so that the concentration per milliliter of cells rises to several hundred times the concentration in the medium. These concentrations can be explained in terms of adsorption or in terms of a pump mechanism that transfers the amino acids to an impermeable region of the cells. Several difficulties promptly appear in both models. Adsorption can hardly be the sole mechanism involved, for the following reasons:

1. The quantity of material held within the cells is very large. Several hundred micromoles per gram dry cell of an amino acid can be held, a quantity which is roughly one-third of the number of RNA nucleotides or one-twentieth the number of protein-bound amino acids in the cell. The adsorption of such large quantities seems unreasonable.

2. The interference between similar amino acids does not have the characteristics expected from competition for adsorption sites.

3. Adsorption processes would not be expected to be sensitive to the osmotic strength of the medium.

The pump model also has serious difficulties:

1. An appreciable fraction of the cell is accessible to amino acids by free diffusion; consequently, the pump cannot be considered as simply working to maintain a concentration gradient across the cell wall.

2. An energy source is required to build up the concentration within the cells, but not to maintain an existing concentration. Exchange continues, however, in the absence of an energy source. Therefore, the

barrier that maintains the concentration is by no means impermeable, and the pump would have to continue to operate without an energy source to maintain the concentration gradient. Without the energy source it could not build up even a much lower concentration.

3. The release of amino acids by osmotic shock does not have the characteristics that would be expected if the process involved bursting a membrane.

4. It is difficult to see how the maximum capacity of a region within a membrane could increase when the osmotic strength of the medium rises.

In the face of these difficulties with both the adsorption and the pump models it seems advisable to attempt the construction of a new model that will meet more of the requirements. In mammalian tissue it is the microsome fraction that is the first to incorporate amino acids. Furthermore, the active elements of the microsome fraction are particles consisting of approximately one-half protein and one-half nucleic acid. Similar particles are found in *E. coli*. If all the RNA of the cells is located in particles that are 50 per cent RNA, then about 30 per cent of the cell mass would be found in the particles. Since about this fraction of the cell is accessible by free diffusion to water, but not to amino acids, and since the amino acids might be expected to localize in regions where protein synthesis occurs, it seems reasonable to postulate that the amino acids of the pools may be trapped within the three-dimensional framework of particles.

Not enough is known of the physical chemistry of these organized networks of nucleic acid and protein to predict definitely what their properties might be, particularly while they are still within the cytoplasm of the cells. It does not seem too unreasonable, however, to suppose that they might exhibit the observed sensitivity to the osmotic strength of the medium. Such structures could provide the needed capacity to hold amino acids, and a degree of specificity might result from

the configuration of their interior spaces.

The idea of such a structure as the basic unit for growth is, of course, not new. The feature that has been added is that these particles may also concentrate the precursors of protein and nucleic acid. If this hypothesis turns out to be true, it may be possible to study some of the characteristics of the particles as they exist within the cells by observing which compounds can or cannot prevent osmotic shock. At this stage, however, the main virtue of the model is that it stimulates new experiments.

#### TORULOPSIS UTILIS

When *Torulopsis utilis* is grown on fructose as sole source of carbon and energy, a large amino acid pool containing 13 per cent of the cell carbon is formed. Kinetic studies describing the steady-state flow of endogenous carbon in the *T. utilis* revealed that this pool of nonprotein amino acids was a direct intermediate in the synthesis of proteins (see Year Book No. 54, 1954-1955, pp. 80-84). Once amino acids are incorporated in the pool, exogenous amino acids have little or no effect upon the internal conversion of these pool amino acids or upon the rate of their incorporation into proteins. It was postulated that an association of a pool amino acid with a macromolecule could account for this behavior. The formation of such a complex would presumably require energy, and it was observed that pool formation ceased in the absence of fructose. The complex seemed reasonably stable, since such materials as alcohol or 5 per cent TCA were required for extraction of pool amino acids whereas distilled water, saline, or medium was ineffective.

The above kinetic studies showed that, in addition to the transfer of amino acid carbon from pool to protein, a small quantity of pool carbon of the TCA-soluble pool was also transferred to the nucleic acids of the cell. The nucleic acid precursor was extremely small in quantity, and its presence was often obscured by the large amounts of amino acid carbon flow-

ing through the pool. To avoid difficulties encountered in measuring these compounds, labeled nucleic acid bases were used as supplements to the synthetic medium, and their flow from pool to nucleic acid end products was determined.

#### Pools of Nucleic Acid Bases

*Pool formation with C<sup>14</sup> adenine.* When C<sup>14</sup> adenine was added to a culture of cells growing exponentially in medium containing nonradioactive fructose, radiocarbon was rapidly incorporated by the cells. Figure 48 shows the time course of incorporation obtained in experiments using the porous collodion membrane filter technique described above. In this experiment only 0.18 millimicromole of adenine was present per milliliter of medium, and, as shown in figure 48, the exogenous supply of adenine was almost completely incorporated by the time the cellular mass had doubled. At first the rate of incorporation into the cold-TCA-soluble fraction was more rapid than incorporation into the fraction containing the nucleic acids (TCA-precipitable fraction), which, after a slight lag, took up the adenine carbon at a constant rate until the supply in the medium approached exhaustion. These results suggest that the exogenous adenine is first incorporated into the TCA-soluble fraction before incorporation into the nucleic acid fraction of the cells. Figure 49 shows the quantity of adenine taken up in micromoles per gram dry weight of cells.

*Transfer of pool adenine to nucleic acid adenine.* Since the incorporation of adenine carbon into the cold-TCA-soluble fraction precedes the incorporation into the precipitable fraction, cells containing most of the radioactivity in the cold-TCA-soluble fraction can be prepared by briefly immersing exponentially growing cells in medium containing C<sup>14</sup> adenine and transferring them, after washing, to medium containing no radioactive supplements. The flow of the labeled cold-TCA-soluble carbon to the nucleic acid fraction can then be followed. An 8-minute immersion

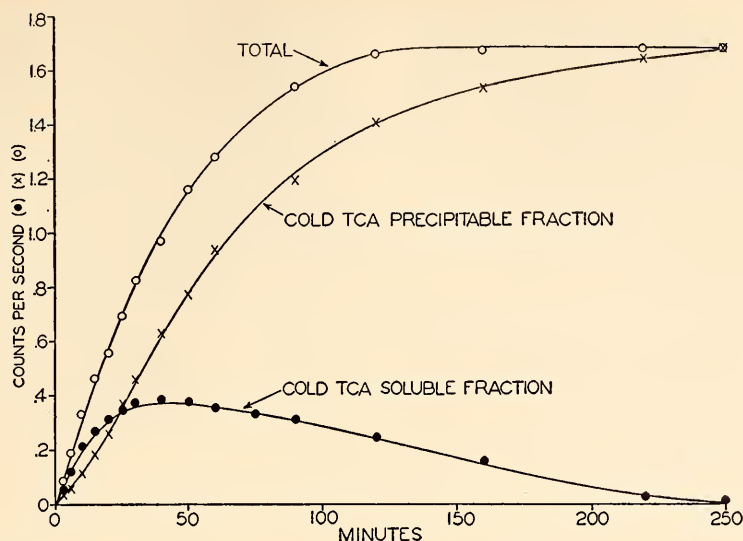


FIG. 48. Time course of incorporation of trace quantities of  $C^{14}$  adenine by exponentially growing *T. utilis*.

of cells in medium containing radioadenine produces cells containing more than 60 per cent of the incorporated carbon in the cold-TCA-soluble fraction. This labeled material rapidly leaves the cold-TCA-soluble fraction, as shown in figure 50, and

appears at the same rate in the hot-TCA-soluble fraction. Little, if any, of the incorporated carbon is lost from the cells during this transformation. It may be concluded that the formation of nucleic acid adenine occurs via a metabolic pool of adenine carbon. These interrelations between the adenine carbon in the cold-TCA-soluble fraction and end-product adenine are similar to those observed with

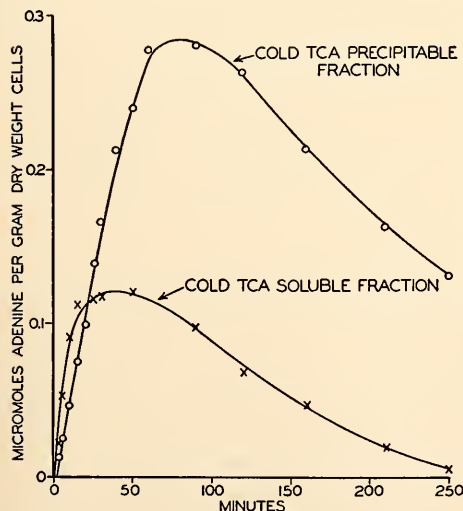


FIG. 49. Micromoles adenine incorporated per gram dry weight cells. Calculations made from data shown in figure 48, assuming that all incorporated carbon remained radioadenine.

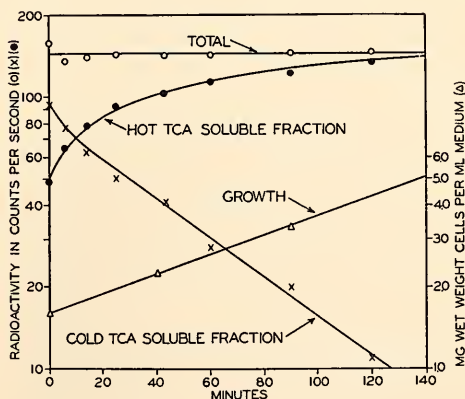


FIG. 50. Kinetics of the transfer of pool carbon to nucleic acid during exponential growth. Carrier-free  $C^{14}$  adenine was used to prelabel the cells.



pool amino acids and protein incorporation.

*Expansion of pool sizes.* When trace levels of  $C^{14}$  adenine are used to supplement the medium (figs. 48, 49, 50) there is insufficient adenine to supply the needs of the growing cells, and endogenous carbon derived from the  $C^{12}$  fructose furnishes most of the carbon necessary for nucleic acid adenine synthesis.

Figure 51 shows the time course of incorporation of  $C^{14}$  adenine when the ex-

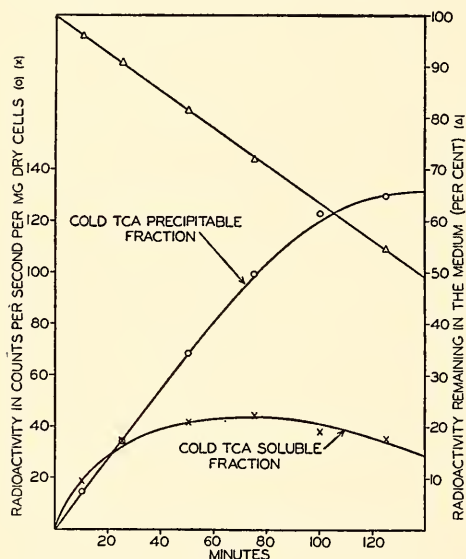


FIG. 51. Time course of incorporation of  $C^{14}$  adenine by exponentially growing *T. utilis* in medium containing high concentrations of adenine.

ogenous concentration was high (0.52  $\mu\text{mol/ml}$  medium). Adenine was so rapidly incorporated that the quantity in the medium (fig. 51) rapidly dropped, and this decreasing concentration was reflected by a decreasing rate of incorporation into the adenine pool. A more dramatic presentation of pool-size variation as a function of exogenous adenine is shown in figure 52. This figure represents the maximum pool size observed in a series of kinetic experiments as a function of the initial adenine content of the medium. No upper limit of adenine pool was reached

even at the higher concentrations of exogenous adenine. Experiments at the higher concentrations are difficult because of the insolubility of the adenine and the great reduction of the specific radioactivity.

*Conversion of adenine carbon to guanine.* It has been established by Kerr, Seraidarian, and Brown that in *T. utilis* exogenous adenine is converted to adenine and guanine of the RNA. It was not surprising, therefore, to observe, upon chromatographic examination of the hot-TCA-soluble fractions of cells grown upon both low and high concentrations of exogenous

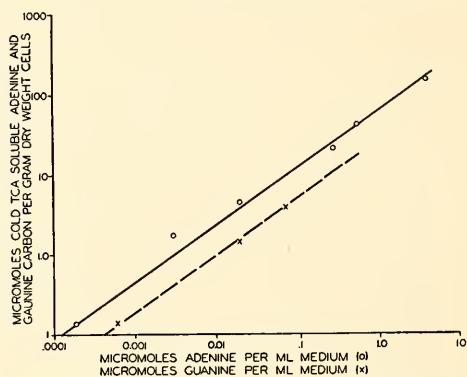


FIG. 52. Expansion of the size of purine pools. The data shown represent the maximum quantity of  $C^{14}$  adenine carbon incorporated into the cold-TCA-soluble fraction as a function of the initial exogenous concentration of  $C^{14}$  adenine. Calculations assume that all the incorporated carbon remained adenine. Similar data are shown for the expansion of guanine pool with increasing concentrations of exogenous  $C^{14}$  guanine.

$C^{14}$  adenine, that these bases both became radioactive. The radioguanine, however, appeared later than radioadenine in the nucleic acids. Table 18 shows the time course of incorporation of carbon from adenine into the guanine of the nucleic acids of the cells. Adenine and guanine were the only regions on the chromatograms that contained radioactivity.

Chromatographic analysis of hydrolyzates of the cold-TCA-soluble fraction of cells grown on  $C^{14}$  adenine showed the same characteristics as described above for



the hot-TCA-soluble fraction. Both  $C^{14}$  adenine and guanine appeared on the chromatograms, the radioactivity mainly occurring at first as adenine and, as later samples were obtained, in both adenine and guanine. Obviously, in these cells, exogenous adenine is taken into the pool; part of the pool adenine is subsequently converted to pool guanine; both are eventually incorporated into the nucleic acids of the cells.

TABLE 18

CONVERSION OF  $C^{14}$  ADENINE TO  $C^{14}$  GUANINE BY EXPONENTIALLY GROWING *T. utilis*

Time (min)	Mg wet wt cells/ml medium	Total radioactivity in hot-TCA-soluble fraction (counts/sec)	Per cent of total radioactivity of hot-TCA-soluble fraction found as guanine
10	1.27	15	26.3
30	1.35	22	34.5
100	1.76	150	39.6
150	2.37	310	44.0
240	3.40	710	45.0

*Isotopic competition results.* Additional support for the conclusion that pool adenine is converted in part to pool guanine before incorporation into nucleic acid is obtained through a study of the kinetics of adenine pool formation in the presence of guanine. Figure 53 shows the effect of adding  $C^{12}$  guanine to a culture growing in medium containing  $C^{14}$  adenine. At the time of addition of the guanine, the concentration of the exogenous  $C^{14}$  adenine was approximately equal to that of the added guanine. Upon the addition of the guanine the rate of incorporation of  $C^{14}$  adenine carbon into the cells was reduced. The cold-TCA-precipitable fraction also showed a reduction in the rate of incorporation.

*Pool formation with  $C^{14}$  guanine.* Results similar to those observed with adenine are obtained when  $C^{14}$  guanine is used as the tracer material. Figures 54 and 55 show the rapid incorporation of  $C^{14}$  gua-

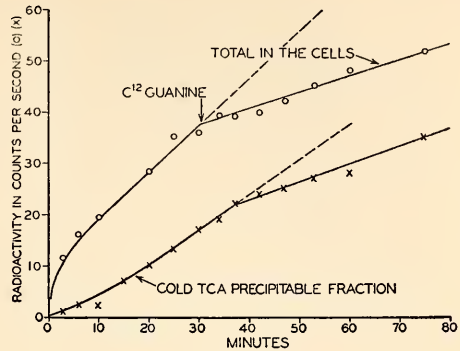


FIG. 53. Kinetics of  $C^{14}$  adenine incorporation in the presence of  $C^{12}$  guanine. At time = 30 minutes, the exogenous concentration of  $C^{14}$  adenine and  $C^{12}$  guanine was 0.016 and 0.018 micromole per ml medium, respectively.

nine. The cells soon deplete the medium of exogenous guanine and are completely dependent upon the endogenous flow of carbon from fructose for the synthesis of nucleic acid guanine. After this brief pulse of externally supplied guanine, the cold-TCA-soluble fraction, which was the first to become radioactive, rapidly loses its radioactivity. The cold-TCA-precipitable fraction, however, continues to gain in radioactivity until the pool is depleted.

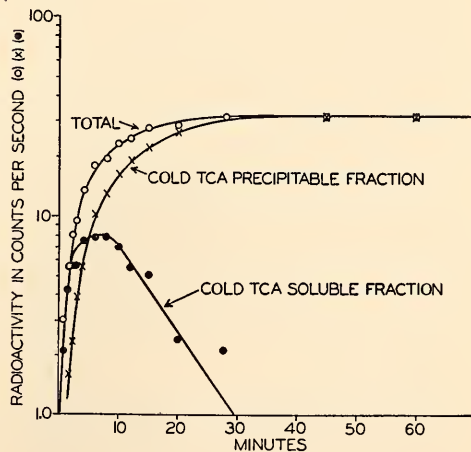


FIG. 54. Time course of incorporation of trace quantities of  $C^{14}$  guanine during exponential growth. Guanine concentration at time = 0 was 0.0005 micromole per ml medium.

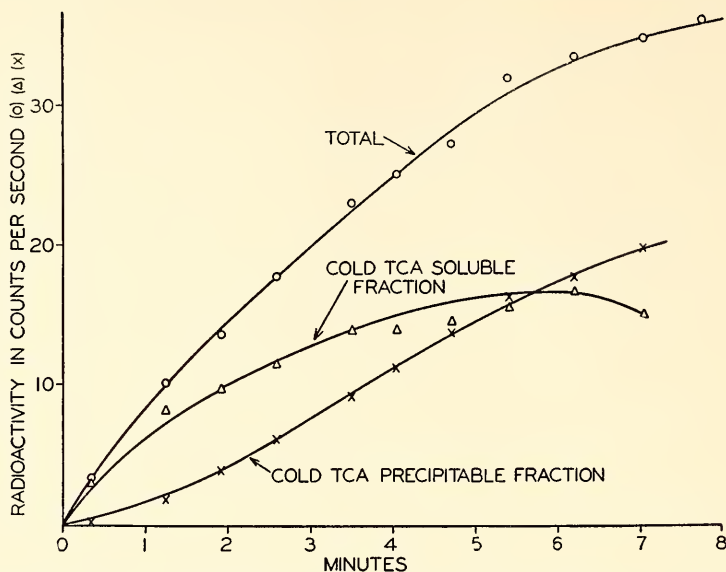


FIG. 55. Short-term experiment showing early kinetics of incorporation of trace quantities of  $C^{14}$  guanine. Initial guanine concentration 0.0009 micromole per ml medium.

When higher levels of exogenous guanine are present, the pool size increases, and as a consequence the length of time during which the pool remains radioactive also increases. Guanine is much less soluble than adenine, and supplements exceeding its solubility in the medium were not used. No upper limit of the guanine pool was ever observed. Figure 52 shows the values of the pools obtained with increasing exogenous concentrations in comparison with those obtained when adenine was used as the tracer.

Chromatographic analysis of hydrolyzates of the cold-TCA-soluble and -precipitable fractions showed guanine as the sole radioactive constituent, regardless of the external guanine concentrations used. Guanine is not readily converted to adenine in the pool by these exponentially growing cells.

Figure 56 demonstrates that the addition of  $C^{12}$  adenine has little or no effect upon the incorporation of  $C^{14}$  guanine. Therefore, exogenous guanine is incorporated into the cold-TCA-soluble guanine pool without any competitive effects from guanine derived from exogenous adenine.

*Tests with other nucleic acid compounds.* When  $C^{14}$  cytidylic and  $P^{32}$  and  $C^{14}$  uridylic acids were used as supplements, no radioactivity was incorporated by the cells. No diminution was observed in the exponential growth rate upon the

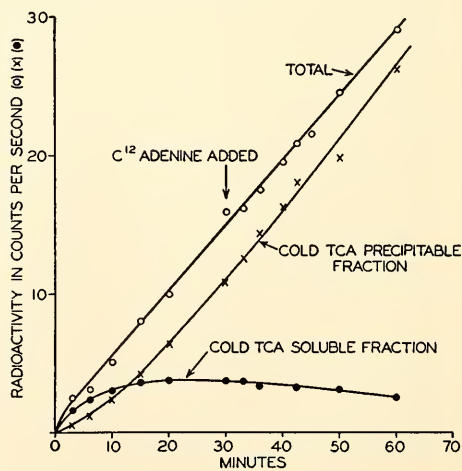


FIG. 56. Kinetics of  $C^{14}$  guanine incorporation in the presence of  $C^{12}$  adenine. At time = 30 minutes, the exogenous concentration of  $C^{14}$  guanine and  $C^{12}$  adenine was 0.065 and 0.053 micromole per ml medium, respectively.

addition of the supplements. The specific radioactivity of these compounds was sufficiently high so that trace levels of incorporation should have been observed had these compounds been usable or degraded to usable products. In no case was there detectable radioactivity in the cells even after several generations of cellular growth in the radioactive medium. This inability of the more complex forms to enter into the biochemistry of the metabolic pool has not been explained.

Figure 57 presents some of the conclusions that have been reached about nucleic

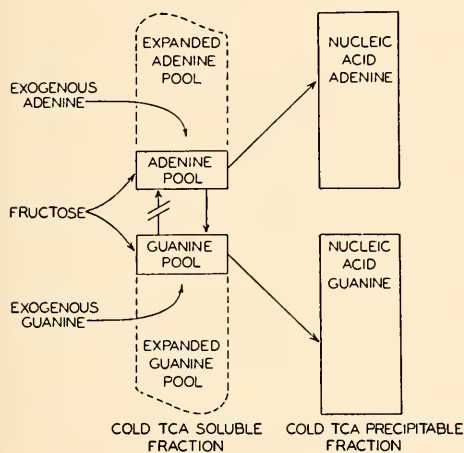


Fig. 57. Flow of purine carbon through metabolic pools in the synthesis of nucleic acid.

acid synthesis in *T. utilis*. Clearly, a metabolic pool of purine compounds serves as precursor material for the synthesis of nucleic acid guanine and adenine. This pool is in many ways similar to the amino acid pool through which amino acid carbon flows in the synthesis of proteins. As in the amino acid pool, where amino acid conversion occurs before protein incorporation, the conversion of pool adenine to pool guanine occurs before guanine is incorporated into nucleic acid. Similarly, as shown in figures 48 and 54, the incorporated pool adenine and guanine can be completely transferred to their end products. At low levels of exogenous supplements of guanine and adenine there is

little or no loss of pool material to the medium. Distilled water, saline, or C medium washes do not remove pool material from the cell. Pool adenine serves as precursor material for pool guanine, nucleic acid adenine, and nucleic acid guanine. Pool guanine is not converted to pool adenine or to nucleic acid adenine.

With increasing external concentrations of guanine and adenine the pool size increases, and at any exogenous concentration these bases are always observed in the pool in quantities far exceeding the medium concentrations. In fact, at the highest levels of exogenous adenine used (fig. 52), the pool incorporated 156 micromoles of adenine carbon per gram dry weight of cells. About 40 per cent of this carbon was contained as guanine, the remainder as adenine. If a density of 1 is assumed for the cells, the pool adenine corresponded to a concentration of 23  $\mu\text{mol}$  of adenine per ml of wet cells. This concentration is 3 times the reported solubility of adenine in water at 25° C. The guanine contained in the pool makes matters even worse. There were 15  $\mu\text{mol}$  of guanine per ml of wet cells. The solubility of guanine in water is only 0.03  $\mu\text{mol}/\text{ml}$ . The pool guanine is 500 times more concentrated than a saturated water solution. *T. utilis*, however, is not just a sac of water; 25 per cent of the cellular volume is already occupied by the solids of the cell. One conclusion to be drawn from such calculations is that pool guanine and adenine are not free compounds in the cytoplasm of the cell. Increased solubility could come from the association of the adenine and guanine with other molecules in the cell. The quantity of pool adenine and guanine found with the highest exogenous concentration of adenine almost equals the quantity of nucleic acid adenine and guanine in the cells. If nucleic acids were forming a complex with these bases, it would be difficult to see how the amino acid pool could also form a nucleic acid complex.

*Relation between the phosphorus and purine pools.* The kinetics of formation of

a phosphorus pool in *T. utilis* and its utilization for nucleic acid and lipid synthesis have been previously described (see Year Book No. 54, 1954-1955, pp. 80-84). This pool contains one-third of the phosphorus of the cell (8.2 mg/gr dry weight). Chromatograms of the cold-TCA-soluble fraction from yeast cells grown in C medium and radiophosphorus were very similar to those from *E. coli* (see fig. 41). In each case 50 per cent of the phosphorus is found as orthophosphate and most of the remainder is distributed among compounds having chromatogram radiofrequencies corresponding to those observed for various nucleotides.

Since the purine pool could be enormously expanded upon the addition of exogenous adenine or guanine, experiments were performed to ascertain whether a corresponding increase in the phosphorus of the pool also occurred. Figure 58 shows that the rate of incorporation of radiophosphorus was not altered by the addition of large amounts of adenine. Chromatograms of the cold-TCA-soluble fraction obtained from cells removed just before, and also 30 minutes after, the addition of the adenine showed no significant differences in the distribution of the radiophosphorus. No free adenine was observed on the chromatogram of the early sample; the later sample contained a large quantity of adenine which was readily detected by ultraviolet-light absorption. No increase in the amount of purine nucleotide was found. The adenine in the expanded pool was not present as a purine nucleotide.

Thus the yeast cell contains two adenine pools. Both are used for nucleic acid synthesis. The expanded pool may lose some of its carbon to the medium. Little loss or exchange occurs in the nucleotide pool.

The nucleotide pool may be specifically labeled by adding small quantities of  $C^{14}$  adenine to the medium. This adenine does not form an appreciable pool of nonphosphorylated adenine, but rapidly becomes a part of the nucleotide pool. The rate of transfer of the carbon of a pre-

labeled nucleotide pool to nucleic acid is the same whether exogenous adenine or guanine is present or not, as is shown in figure 59.

Also, the rate of appearance of radiocarbon in the nucleic acids of the cells is not affected by the expansion of the nonphosphorylated pool. The exponential rate of loss of nucleotide pool carbon and the appearance of most of this carbon in the nucleic acid indicate that the radiocarbon in the pool is mixed with the nonradioac-

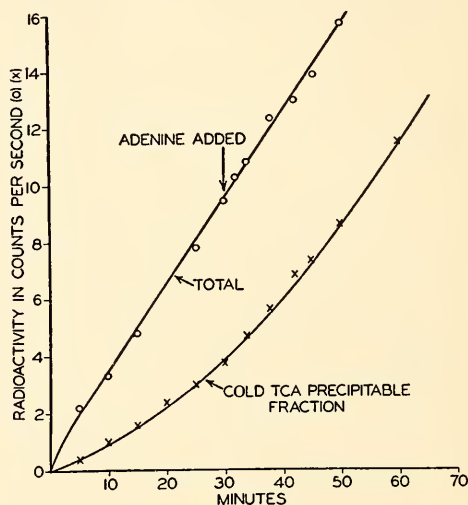


Fig. 58. Kinetics of incorporation of radiophosphorus during expansion of the adenine pool.  $C^{14}$  adenine added at a concentration of 1 micro-mole per ml medium at time indicated.

tive precursor of nucleic acid. If the expanded nonphosphorylated adenine or guanine pools were the direct precursor material, a dilution of the radiocarbon by the expanded pool should occur, and consequently there should be a difference in the rates of transfer between the cells with and without the expanded pools.

Such is not the case. Mixing of the two pools does not occur; therefore, it is the phosphorylated pool and not the expanded pool that serves as the direct precursor material. The nonphosphorylated pool may compete with fructose carbon in providing



material for the formation of the nucleotide pool, but the carbon of either source must flow through the phosphorylated pool in order to be used for nucleic acid synthesis. Thus, two processes are involved in the incorporation of exogenous adenine: one is the incorporating mechanism that builds up materials to concentrations exceeding that of the external environment; the other is the process in which selection and

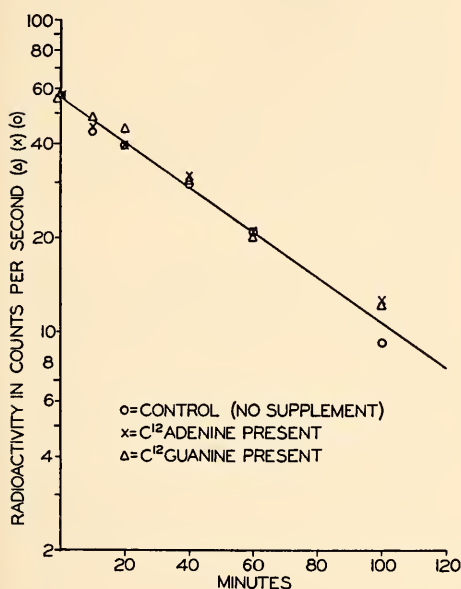


FIG. 59. Loss of  $C^{14}$  adenine carbon from the cold-TCA-soluble fraction. Data obtained from cells briefly immersed in medium containing  $C^{14}$  adenine (carrier-free) and transferred after washing to nonradioactive medium containing supplements  $x$  of  $C^{12}$  adenine (0.1 mg/ml medium);  $\Delta$ ,  $C^{12}$  guanine (0.005 mg/ml medium); and  $O$ , control (unsupplemented C medium). At time = 0, 80 per cent of the incorporated carbon was in the cold-TCA-soluble fraction, and at the end of the experiment the majority of this radiocarbon was contained in the hot-TCA-soluble fraction. There was little loss of pool radiocarbon to the medium.

alteration occur, furnishing the proper compounds for the macromolecules. The two different pools of adenine described above can thus be used to investigate the details of each process independently.

### Amino Acid Pools

*Pool formation with  $C^{14}$  amino acids.* The kinetics of incorporation of amino acid carbon by exponentially growing *T. utilis* has also been investigated. Figure 60 shows the time course of incorporation of tracer levels of  $C^{14}$  glutamic acid. The exogenous glutamic acid was quickly incorporated, the radiocarbon appearing first in the cold-TCA-soluble fraction. As the exogenous supply was exhausted, this fraction lost radioactivity to the precipitable fraction. When higher concentrations of  $C^{14}$  glutamic acid were used, the radioactivity of the glutamic pool was maintained until there was a significant reduction in the concentration of the exogenous glutamic acid.

These kinetic results resemble those obtained in the investigation of purine incorporation. Similarly, expansion of the amino acid pool occurs upon supplementing the C medium with high concentrations of amino acids. Table 19 shows the quantity of threonine carbon contained in the cold-TCA-soluble and -precipitable fractions as a function of increasing threonine concentrations. Chromatograms of the pool and protein fractions showed that the incorporated radiocarbon in the pool was threonine; that in the protein, threonine and isoleucine. The ratio of threonine to isoleucine in the proteins of the cells grown at the highest concentration of threonine was approximately 3 to 1.

*Endogenous formation of amino acid pools from fructose.* The formation of amino acid pools in cells grown in medium containing  $C^{14}$  fructose as the sole carbon source has previously been described. Table 20 shows the steady-state distribution of pool and protein amino acids obtained from such cells. In the amino acid pool there is a large spread in amino acid concentrations not found in the protein fraction. The pool content of each amino acid is always less than its protein counterpart. A comparison of tables 19 and 20 shows that the pool formed from

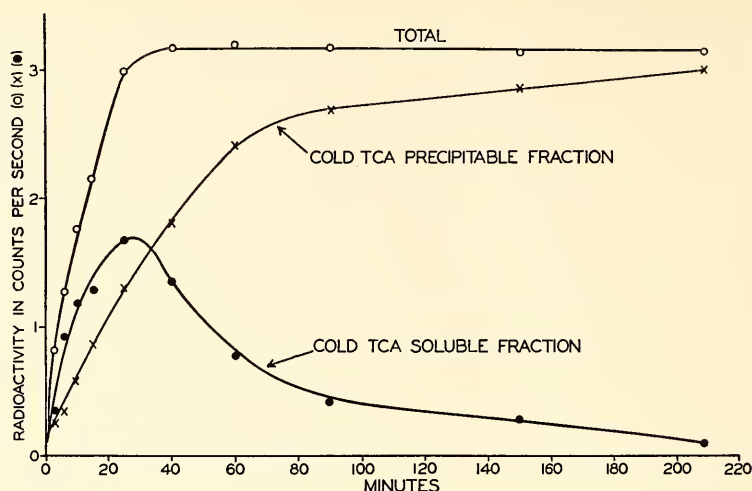


FIG. 60. Time course of incorporation of tracer quantities of  $C^{14}$  glutamic acid. At time = 0, the exogenous concentration was 0.002 mg glutamic acid per ml medium.

exogenous amino acids may greatly exceed the endogenous pool formed from fructose. Indeed, when exogenous  $S^{35}$  methionine at high concentrations is used to supplement the medium, the quantity of labeled cystine and methionine in the expanded pool is equal to the protein content of these amino acids. In the pool formed from fructose the methionine and cystine content are extremely low.

There is another similarity in the amino

acid and purine pools. We have previously shown that, when the amino acid pool is specifically labeled with a brief pulse of  $C^{14}$  fructose, the rate of transfer of the carbon of these prelabeled amino acids to protein is the same whether high concentrations of exogenous amino acids are present or not. Furthermore, the conversion in the pool of one prelabeled amino acid to other amino acids occurs at the

TABLE 19  
DISTRIBUTION OF  $C^{14}$  THREONINE CARBON AMONG  
POOL AND PROTEIN AMINO ACIDS \*

CONCENTRATION OF EXOGENOUS $C^{14}$ THREONINE ( $\mu$ M/ML MEDIUM)	MICROMOLES THREONINE PER GRAM DRY WEIGHT CELLS	
	Cold-TCA- soluble fraction	Cold-TCA- precipitable fraction †
50.0	442	520
8.4	327	446
6.7	268	328
5.0	190	272
3.4	104	149
1.7	32	82
0.8	9	59

\* Cells grown from light inoculum to about 2.0 mg wet weight of cells per ml medium.

† Calculated on the basis that all the radiocarbon incorporated remained  $C^{14}$  threonine.

TABLE 20  
STEADY-STATE DISTRIBUTION OF RADIOCARBON  
AMONG POOL AND PROTEIN AMINO ACIDS \*

Component	Pool quantity of compound ( $\mu$ mol/gr dry)	Protein quantity of compound ( $\mu$ mol/gr dry)
Isoleucine-leucine	117	785
Lysine	24	625
Glutamic acid	290	640
Aspartic acid	9	762
Valine	65	512
Alanine	240	695
Threonine	8	455
Serine	8	600
Proline	7	287
Arginine	63	210
Glycine	108	488
Per cent accounted for	87	85

\* Data obtained from cells growing exponentially in C medium containing  $C^{14}$  fructose.

same rate when high concentrations of exogenous amino acids are present as when no supplements are added.

In the case of the purine pools it was possible to differentiate between the expanded pool and the pool formed from fructose, as the latter was always phosphorylated. It is suggested that the fructose-formed amino acid pool, by analogy with the phosphorylated purine pool, is different from the expanded pool of amino acids. The nature of this difference is not known. There is insufficient phosphorus in the cells to phosphorylate all the amino acids in the fructose-formed pool. Some alteration of these amino acids is necessary, however, to account for the failure of mixing of the amino acids in the two pools.

### Conclusions

The above studies permit us to draw some tentative conclusions regarding the synthesis of macromolecules in *T. utilis*. In the synthesis of nucleic acid we are able to recognize at least two precursor pools containing purine bases. From studies investigating the incorporation of exogenous adenine and guanine it was shown that an expandable pool of unphosphorylated bases is contained within the cell. The quantity of material concentrated in this pool is variable, and the size of the pool is dependent upon the external concentrations of the purine used.

Once concentrated within the cell, the bases in this pool furnish material for a second pool. Here the bases are phosphorylated, and the appropriate selection and conversion of nucleotides occurs before nucleic acid incorporation.

In addition to their functional differences, other qualities differentiate the two pools. The expandable pool may contain guanine and adenine in quantities large enough to be insoluble were they free bases at these concentrations in the medium. This fact suggests that some alteration of the molecules occurs during the concentrating process. When such pools are expanded, the requirements for *de novo* syn-

thesis from fructose to form materials for the pools are greatly reduced. The purine bases of the expanded pool cannot be directly incorporated into nucleic acid, however, without first passing through the nucleotide pool.

In an analogous manner there appear to be two pools of amino acids that are used for protein synthesis. The endogenous amino acid pool formed from fructose is large, containing 13 per cent of the carbon in the cell. Despite its size and its direct relation to the synthesis of proteins, additional quantities of amino acids may be concentrated in the cell when exogenous amino acids supplement the medium. This expandable pool does not mix with the large fructose-formed pool but may supply amino acid carbon for its maintenance. The mechanism concentrating the amino acids in the expandable pool is unknown, and the alteration of the amino acids in the fructose-formed pool producing the difference between the two pools has not been detected. Upon extraction with cold TCA or alcohol, free amino acids are obtained from both pools. Conversion of one amino acid to another occurs in the fructose-formed pool, and it is from this pool that amino acids are withdrawn for protein incorporation.

Although, in many respects, the conclusions reached are similar to those obtained from the extensive studies of *E. coli* described above, slight differences exist. In *T. utilis*, the pools are larger and materials in them more firmly retained. These cells are less sensitive to osmotic shock. Despite these quantitative differences, it is apparent that the over-all pattern of macromolecule synthesis closely resembles the patterns observed in our studies with *E. coli*.

### RADIOACTIVE TRACER STUDIES ON HIGHER ORGANISMS

#### *Radioactive Tracer Studies on Hydra*

*Hydra* is a fresh-water, many-celled animal. Essentially, it is a double-walled digestive sac, whose oral end is fashioned into a ringlet of mobile, food-capturing



tentacles. These fascinating creatures are used extensively as teaching aids in general biology courses. Few students or professional biologists realize, however, that researches on these organisms by Trembley in 1740 proved that animal reproduction without mating could occur and, more revolutionary still, that whole animals could regenerate from isolated parts. In spite of this important role in pushing back the frontiers of biology, metabolic research on *Hydra* was not again seriously considered until W. F. Loomis' recent work, started in 1952, which has brought the laboratory culture of these creatures under precise control.

*Hydra* possesses a number of advantages of microorganisms for investigations of growth, differentiation, and reproduction. *Hydra* can be maintained in simple water solutions, reproduces asexually at an exponential rate when fed on brine shrimp (*Artemia*) larvae, and is metabolically responsive to changes in the composition of the culture medium. Though a multicellular animal, it has only seven main cell types, which are organized into relatively simple systems recognizable both morphologically and biochemically. *Hydra* are large enough (about 40 micrograms wet weight; about 1,000,000 cells) to be worked with individually, yet small enough to be studied as statistically significant populations (e.g., one to ten thousand individuals). Loomis has shown how one of the processes of differentiation, the transformation from asexual to sexual types, can be controlled. Finally, *Hydra* is considered by many biologists to occupy a position at the junction of the evolutionary pathways which are supposed to lead from the Protozoa to the Lower Metazoa and thence toward man. Because of these attributes, *Hydra* seemed a promising subject in which to test some of the tracer techniques developed in our earlier studies on microorganisms. During the past year a number of experiments were carried out to assess the usefulness of *Hydra* for tracer investigations.

When this work was started there was no hint whether *Hydra* could incorporate any ion or molecule directly from its culture medium. The first experiments showed that radioactivity from  $P^{32}$ -labeled inorganic phosphate, from  $S^{35}$ -labeled inorganic sulfate, or from  $C^{14}$ -labeled fructose was incorporated by nonfeeding *Hydra*. The radioactivity was not removed from the organisms upon thorough washing with nonradioactive culture medium, or upon treatment with boiling Bouin's fixative (a mixture of glacial acetic acid, picric acid, and formaldehyde). Since *Hydra* does not normally open its mouth except to ingest or eject particulate matter, it was concluded that *Hydra* was probably permeable to the labeled materials used and could fix experimentally useful amounts of them into chemically stable components of its protoplasm.

When *Hydra* was exposed to a tracer-labeled substance for increasing periods of time, the amount of label incorporated also increased. In the case of  $P^{32}O_4^{=}$ , studies were made of nonfeeding and actively feeding populations. It was found that feeding brine shrimp larvae reduced the uptake of radioactivity to low levels. It was concluded that brine shrimp contain phosphorus compounds that are utilized in preference to  $PO_4^{=}$ ; thus isotopic competition promises to be a useful tool for metabolic studies in *Hydra* as in other forms.

*Hydra* requires a source of food such as the brine shrimp for normal growth and reproduction. Brine shrimp, however, are as chemically complex as other living things, and they introduce an uncontrolled variable of great complexity into the experimental system. It would be desirable to eliminate this variable by culturing *Hydra* in a chemically defined medium. One step toward deciding what is useful in such a medium is to ascertain what kinds of organic chemicals can be utilized for the synthesis of *Hydra* components. An experiment in which a population of nonfeeding *Hydra* was allowed





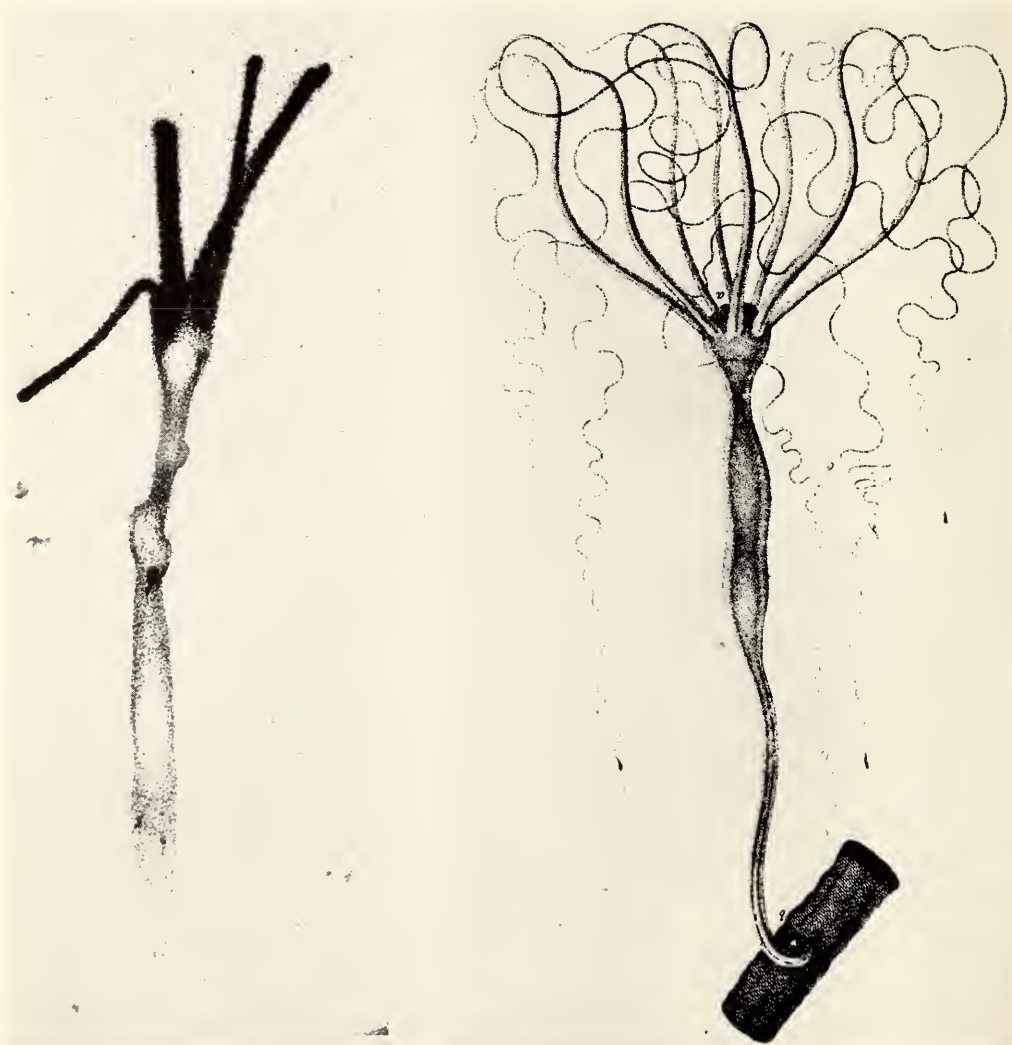


FIG. 61. Radioautograph of a  $C^{14}$ -labeled *Hydra* (left), and a reproduction of one of Lyonet's illustrations from Trembley's monograph of 1744 (*Mémoires, pour servir à l'histoire d'un genre de polypes d'eau douce à bras en forme de cornes*. Leide, Verbeek). The two *Hydra* shown are different species. Note the intense labeling of the tentacles and the clearly indicated batteries of stinging cells.

to metabolize randomly  $C^{14}$ -labeled amino acids was revealing. In a 24-hour period, 21 *Hydra* incorporated essentially all the radioactivity (equivalent to approximately 2  $\mu$ g of carbon) supplied. Paper chromatograms of the amino acids derived from the labeled organisms by acid hydrolysis showed that every *Hydra* amino acid contained  $C^{14}$ . The relative amounts of  $C^{14}$  differed among the amino acids, and the  $C^{14}$  distribution differed also from that in the material supplied. It is evident, therefore, that *Hydra* can take up amino acids from the culture medium and convert them into protein. Some amino acids are taken up more efficiently than others, and conversions of some to others can occur. This experiment provides increased hope that a chemically defined culture medium for *Hydra* is a practical goal. A radioautograph of one of the  $C^{14}$ -labeled *Hydra* is shown in figure 61, together with a reproduction of a drawing done by Lyonet to illustrate Trembley's 1744 monograph.

Radioautographs of labeled *Hydra* were prepared from time to time during these experiments. They showed that, as the time of exposure of *Hydra* to a labeled compound was increased, the distribution of radioactivity among the various tissues was altered. Thus,  $P^{32}O_4^{=}$  contributed radioactivity first to the spermaries whenever they were present, next to the region below the mouth and to the circle of gland cells in the basal disk, then to the tentacles, and finally to the stalk cells and the slime secreted into the culture fluid.  $S^{35}O_4^{=}$  and  $C^{14}$  fructose, on the other hand, labeled first the slime, then the tentacles—especially the batteries of stinging cells—the remaining cells rich in protoplasm, and finally the vacuolated stalk cells. The patterns of differential labeling exhibited in the radioautographs of individual organisms were quite consistent.

It was possible, therefore, to concentrate most of the  $P^{32}$  incorporated by an adult *Hydra* into body regions reasonably dis-

tant from the site at which a new individual would develop through the asexual budding process. A number of individuals were labeled in this way, washed free of unincorporated  $P^{32}$ , and cultured in non-radioactive media on brine shrimp. The radioactivity from the  $P^{32}$  in each organism was counted daily. When a bud had developed and became free from its parent it was isolated and counted. The original parents continued to bud, and the isolated first buds matured and gave rise, in turn, to offspring. The experiment was discontinued at the third asexual generation. It was found that a nearly constant, large proportion (25 to 30 per cent) of parental  $P^{32}$  was contributed to the offspring of each asexual generation. Radioautographs of the offspring showed that the  $P^{32}$  was now relatively evenly distributed over the whole organism. These findings demonstrate what has long been obvious: namely, asexual reproduction, in contrast to sexual reproduction, involves relatively large amounts of parental protoplasm. But they also raise questions about the mechanisms of this transfer (for example, whether by cellular division, or by parent cell breakdown and resynthesis of the products into bud cells), and they provide a means for estimating the relative importance of the flows of materials from the parent and from exogenous sources into the newly developing organism.

The results and conclusions discussed above are tentative and need to be confirmed. Nevertheless, this work, which was carried out in collaboration with Dr. Alan A. Boyden at Rutgers University, has already shown that the neglected "polype" (Trembley, 1744) *Hydra* is eminently well suited for tracer work on the problems of growth, differentiation, and reproduction.

#### *Radioactive Tracer Studies on the Mouse*

The biophysics group has profited by collaboration with Drs. L. B. and J. B.

Flexner in their studies of protein synthesis in newborn mice. Their interest lies in observing the rate of protein synthesis as influenced by the total growth rate and the state of functional development of organs. The newborn mouse is a favorable object for study, as much of the development takes place after birth.

After the subcutaneous injection of  $C^{14}$  glucose and  $C^{14}$  amino acids, the rate of appearance of  $C^{14}$  compounds in the blood, liver, and brain cortex has been observed. When  $C^{14}$  glucose is injected, the  $C^{14}$  glucose of the blood rises and falls rapidly, with a peak about 10 minutes after injection.  $C^{14}$  amino acids appear in the cold-TCA-soluble fraction of the blood, brain, and liver with a peak of about 1 hour. The protein-bound amino acids both of the brain and of the liver rise slowly and steadily.

These experiments could not determine the source of amino acids observed in the organs. For example, the free amino acids observed in the brain (although they were at much higher concentrations than in the blood) might have been synthesized elsewhere and concentrated from the blood. Glutamic acid was then injected, and it was found that the level in the brain was only slightly higher than that in the blood. Evidently, glutamic acid is not highly concentrated by the brain or liver from the blood. The high levels previously observed in the brain (derived from a  $C^{14}$  glucose injection) must therefore have been synthesized by the brain tissue itself.

The difference between essential and nonessential amino acids can be demonstrated in several ways. Essential amino acids are those that must be supplied in the diet to maintain normal growth. It might be expected, therefore, that the essential amino acids would be supplied by the blood and not synthesized by the organs. When  $C^{14}$  glucose was supplied,

the radioactivity did not appear in the essential amino acids. They were not synthesized by either brain or liver. In another experiment both essential and non-essential amino acids were injected. The difference between the two classes appeared as a much more rapid incorporation of the essential amino acids into the protein-bound fraction. This incorporation was not delayed by dilution from amino acids synthesized *in situ*. These experiments show that the essential amino acids are indeed essential, even to individual organs.

#### VISITORS

Throughout the year we have had a number of visitors from other laboratories. A conference in honor of Dr. Vannevar Bush was attended by Dr. L. B. Flexner, University of Pennsylvania; S. B. Hendricks, U. S. Department of Agriculture; A. D. Hershey, Department of Genetics, Carnegie Institution of Washington; C. Levinthal, University of Michigan; E. C. Pollard, Yale University; and S. Spiegelman, University of Illinois. Dr. A. C. R. Dean, of Oxford, could not attend the conference but spent two weeks at the laboratory in the spring. Dr. George Mandel, of George Washington University Medical School, was a collaborator in the studies of 6-mercaptopurine. Dr. H. Morovitz, of Yale University, spent a week with us to carry out some analyses of the amino acid composition in various protein fractions. Dr. E. C. Pollard with Dr. C. Woese and Dr. J. Preiss used the cyclotron for two weeks in some radiation studies. Dr. Alex Schrift, of the University of Pennsylvania, spent two weeks in collaborative work on sulfur and selenium metabolism. Mr. T. W. Tuve, of Cornell University, and Mr. C. F. Stroebel, of the University of Minnesota, each participated in the work of the laboratory for several weeks to become familiar with tracer work.



## OPERATIONS AND STAFF

## CO-OPERATIVE WORK OF THE DEPARTMENT

The usual co-operative research work has been continued with foreign and domestic institutions, including the Bernard Price Institute of Geophysical Research (Africa), Brookhaven National Laboratory, Department of Defense, Geological Survey, Geophysical Institute of Huancayo (Peru), Lowell Observatory, National Bureau of Standards, National Science Foundation, Tulane University, Universities of Pennsylvania and Virginia, Mount Wilson and Palomar Observatories, and Cavendish Laboratory (England). We continued our collaboration with the International Union of Geodesy and Geophysics and the International Scientific Radio Union. Our facilities have been used by foreign visitors, some on fellowships of the Institution, from England, Holland, Norway, and Western Australia.

We have continued with the Geophysical Laboratory the investigation of mineral ages by isotope measurements.

Our cosmic-ray program has had continued assistance from the observatories at Cheltenham, Maryland; Christchurch, New Zealand; Climax, Colorado; Godhavn, Greenland; Huancayo, Peru; and Mexico, D. F.

Contracts with the government (no funds involved) are still in force for the investigation of the earth's crust and of cosmic rays, and for the procurement of isotopes for nuclear studies and the measurement of mineral ages.

The Department was cosponsor with the National Science Foundation of a Conference on Theoretical Geophysics held in Washington, D. C., February 1-3, 1956; a grant for the expenses of this meeting was administered by the Department (no overhead charges).

At the request of the National Academy of Sciences, a symposium on solar and terrestrial relations in geophysics was organized together with Dr. John Simpson, of the University of Chicago, who first proposed this conference.

The Department also administers, without charge, two grants from the National Science Foundation for the activities of the NSF Advisory Panel on Radio Astronomy.

Several staff members have continued to serve on panels of the U. S. National Committee, International Geophysical Year, and one is a member of the Executive Committee of that Committee; on June 1, 1956, one member started devoting full time for the National Academy of Sciences to studies concerned with a world data center in connection with the International Geophysical Year. One member completed his service with the Advisory Panel on General Sciences of the Department of Defense and as chairman of the National Academy Advisory Committee to the Federal Civil Defense Administration, but continues as chairman of the Advisory Panel on Radio Astronomy of the National Science Foundation. Two members are on the Committee on Growth of the National Research Council, and one continues to serve on the Advisory Committee to the Federal Civil Defense Administration. One member completed on January 1, 1956, his directorship of the Institute of Radio Engineers for Region III, but continues as chairman of the U. S. A. National Committee, International Scientific Radio Union. One staff member is teaching a graduate course in nuclear physics at the University of Maryland. Another staff member continues his full-time research work for the government.

The Department had representatives at foreign meetings of the Mixed Commission on the Ionosphere, the International Astronomical Union, the Radio Astronomy Symposium, and the cosmic-ray conference of the International Union of Pure and Applied Physics, and also at the joint meeting of the Special Committee on the International Geophysical Year and the subcommission on cosmic-ray intensity variation of the International Union of Pure and Applied Physics.

## ADMINISTRATION AND OPERATION

The publication of the *Journal of Geophysical Research* has been continued, with partial subsidy by the Institution.

Activities of the Department's radio astronomy group have necessitated the rental of parts of one farm, but work has been completed on another farm.

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## STAFF AND ORGANIZATION

### SCIENTIFIC STAFF

*Director:* M. A. Tuve.

#### *Staff Members*

*Geophysics:* L. T. Aldrich, B. F. Burke, J. W. Firor, S. E. Forbush, J. W. Graham, E. A. Johnson,\* H. E. Tatel, G. R. Tilton, E. H. Vestine,† H. W. Wells, G. W. Wetherill.

*Laboratory and Biophysics:* E. T. Bolton, R. J. Britten, D. B. Cowie, N. P. Heydenburg, R. B. Roberts, G. M. Temmer.

*Guests, Associates, Fellows, and Visiting Investigators:* E. H. Creaser, University of Cambridge; A. C. R. Dean, Oxford University, England; W. C. Erickson, University of Minnesota; H. Faul, U. S. Geological Survey; L. B. Flexner and Mrs. J. B. Flexner, University of Pennsylvania; W. K. Ford, Jr., University of Virginia; K. L. Franklin; H. L. Helfer; P. M. Jeffery, University of Western Australia; J. J. Leahy, University of California; H. G. Mandel, George Washington University; B. R. Mottelson, Institute of Theoretical Physics, Copenhagen, Denmark; D. Nordberg, Chr. Michelsens Institute, Bergen, Norway; E. C. Pollard, Yale University; J. Preiss, Yale University; G. Reber; Mrs. I. Z. Roberts; M. Schmidt, University of Leiden, Leiden, Holland; Alex

\* On leave of absence for government work.

† On leave of absence for work for National Academy of Sciences.

Schrift, University of Pennsylvania; C. F. Stroebe, University of Minnesota; M. Sugiura, University of Alaska; D. H. Wilkinson, Cavendish Laboratory, Cambridge, England; C. Woese, Yale University.

### OPERATING STAFF

*Administrative:* M. B. Smith, W. F. Steiner.

*Office and Clerical:* Mrs. C. C. Ator, Mrs. M. Q. Chapin,§ W. N. Dove, C. S. Leonard, Jr., Mrs. A. P. Moffett, Miss H. E. Russell, Mrs. C. W. Windmuller.

*Instrument Shop:* F. J. Caherty, B. J. Haase, L. A. Horton, J. G. Lorz.

*Research Assistants and Laboratory Assistants:* S. J. Buynitzky, J. B. Doak, E. T. Ecklund, Miss E. F. French, P. A. Johnson, C. A. Little, Jr., R. W. Reuschlein, W. E. Scott, Mrs. B. P. Walton.

*Computer:* Miss I. Lange.

*Maintenance:* C. Balsam, C. R. Domton, P. C. Hovgard,§ P. M. B. de Macedo, E. Quade, S. Swankowski.

*Part-Time and Temporary Employees:* Thirteen part-time and temporary employees were engaged during the year, usually for short periods, to assist in the office and laboratory work.

§ Resigned.



## GEOPHYSICAL LABORATORY

*Washington, District of Columbia*

PHILIP H. ABELSON, *Director*

The Geophysical Laboratory experienced a particularly stimulating and productive year. At any time a wide variety of intellectual fare is available for the scientists of the Washington area, and a constant stream of distinguished visitors comes to our Laboratory. This year there were many additional stimuli. The Laboratory was organizer and host for two excellent symposia: the Vannevar Bush Symposium on the Role of Volatiles in Geological Processes, and a Symposium on Metamorphic Facies. At each of these a group of about thirty leading scientists met for three days to consider intensively an important facet of earth science. Professor C. E. Tilley, of Cambridge University, spent January, February, and March with us, and was a vital source of enthusiasm and activity. Our other Research Associates—Dr. W. F. Libby, Professor G. J. F. MacDonald, and Professor H. Ramberg—contributed many interesting new ideas. Seven predoctoral and postdoctoral fellows and visiting investigators have made their contribution to the creation of a stirring atmosphere. Arrangements have been completed for an even larger group this autumn and winter. In an additional interesting experiment, the Geophysical Laboratory conducted a weekly seminar, "Current Topics in Geochemistry and Experimental Petrology," at Johns Hopkins University for a semester. This activity was highly beneficial to both speakers and audience.

At the same time the work of the Laboratory has shown striking progress. Boyd and England have built an apparatus capable of achieving pressures of 60,000 atmospheres at fairly high temperatures (about 700° C) with which they have succeeded in making dense minerals such as might exist deep in the earth. They have conducted reconnaissance experiments on feldspars, feldspathoids, and denser minerals

up to 60,000 bars at 700° C. Transitions have been found to occur in nepheline, leucite, and anorthite; anorthite is common as a component in plagioclase in deep-crystallizing plutonic rocks, and its breakdown at depth might contribute to the Mohorovičić seismic discontinuity. The behavior of nepheline under these high pressures has been determined in detail.

Through the development of a new technique for controlling the oxidation state of iron, Eugster has synthesized pure iron-bearing mica annite and has opened new areas of opportunity in mineral synthesis. He has determined the field of stability of this mica as a function of temperature, oxygen pressure, and water pressure, and has shown that even a small increment of oxygen pressure may drastically lower the breakdown temperature of this mineral.

The co-operative age program being carried on by this Laboratory and the Department of Terrestrial Magnetism has had several triumphs. Perhaps most significant is the emerging realization that when  $K^{40}$ - $A^{40}$  and  $Rb^{87}$ - $Sr^{87}$  ages are measured on many ancient minerals, a concordant age is often obtained. Since the decay schemes and products are completely different, agreement of the two independent ages must be taken as dating a common event.

Abelson has shown that amino acids such as glycine, sarcosine, alanine, and  $\beta$ -alanine can be formed from a variety of "primitive atmospheres." Simulating conditions that might have been present early in the history of the earth, he has subjected gaseous mixtures to electrical discharges. Among the combinations yielding amino acids were  $CO_2-N_2-H_2-H_2O$ ,  $CO-N_2-H_2-H_2O$ ,  $CO_2-NH_3-H_2-H_2O$ , and  $CH_4-N_2-H_2O$ .

Libby has extended his studies of a technique of absolute beta counting. He has discovered a general relation between the beta absorption coefficient and the maximum energy of the radioactivity. The counting technique permits sensitive, absolute assay of low-level samples to about 5 per cent for all beta-emitting isotopes involving single transitions. These isotopes include nearly all those of practical interest to industry and medicine.

Kullerud and Yoder have made the first

determinations of stability of a sulfide mineral under high pressures. Using a new technique which has general applicability, they have studied the upper stability curve of pyrite ( $\text{FeS}_2$ ) and have determined that it is stable below  $815^\circ \text{C}$  at a pressure of 5000 bars. Results of these experiments can be used to explain why primary pyrite occurs in deep-seated granites and amphibolites but not in basalts or rhyolites. These and other contributions are described in greater detail below.

## PHASE EQUILIBRIA AT HIGH PRESSURE

F. R. BOYD, JR., AND J. L. ENGLAND

The study of phase equilibria in mineral systems in the pressure region above 10,000 bars is a rapidly developing field. For the first time it is possible conveniently to explore mineral stabilities at pressures and temperatures equivalent to those existing in the earth at depths as great as 250 km. The results that can be obtained at these pressures are of great significance to both geophysics and petrology.

Knowledge of the deeper parts of the earth's crust and of the mantle below the crust has thus far come largely from seismology. Seismic studies have provided estimates of the velocities of seismic waves in the earth at various depths, and also estimates of the depths at which discontinuities in these velocities occur.

Observed natural velocities can be compared with velocities determined for minerals and rocks in the laboratory. When account is taken of the variation of the experimental velocities with temperature and pressure, it is found that the velocities observed for the mantle below a depth of about 1000 km cannot be approximated by the velocities determined for any known silicates. Above about 1000 km the observed velocities can be approximated by a few dense silicates, including garnets, pyroxenes, and olivines. It is usually inferred that the upper part of the mantle is composed of such silicates. If the deeper part of the mantle has a similar chemical

composition, it must be composed of phases unknown to us at low pressures.

Results of recent extreme pressure studies have suggested that the restrictions commonly placed on the chemical composition of the upper mantle may not be valid. A number of hitherto unknown high-pressure phases corresponding in composition to feldspars and feldspathoids have already been discovered. Others will surely be found as these studies are pursued. Little is now known of the physical properties of these phases, but should they be found to have elastic parameters comparable with those of garnets and olivines, the possible range of chemical composition in the upper mantle may be greatly extended. Inasmuch as many lavas are believed to originate in the upper part of the mantle, this matter is of concern to the petrologist.

High-pressure studies may also contribute to a better understanding of seismic discontinuities in the earth. In the present state of our knowledge, these discontinuities in wave velocities can be explained as either discontinuities in chemical composition or phase changes, or both. It will be most interesting to discover whether the pressures at which phase changes are produced in common silicates in the laboratory correspond to the pressures obtaining at earth levels characterized by major seismic discontinuities.

The petrologist has a stake in phase-

equilibria studies at pressures above 10,000 to 15,000 bars which goes beyond the application of such studies to the problems involved in the generation of lavas. Because of the rapid reaction rates observed in the pressure range above 10,000 bars, petrologically vital systems can be investigated in which, at lower temperatures and pres-

taken the Bridgman apparatus to temperatures up to  $900^{\circ}\text{C}$  by means of an exterior furnace. At high temperature the strength of the apparatus is reduced; for example, at  $700^{\circ}\text{C}$  it can maintain 60,000 atm. The apparatus now in use at this Laboratory follows the design developed by Griggs and Kennedy.

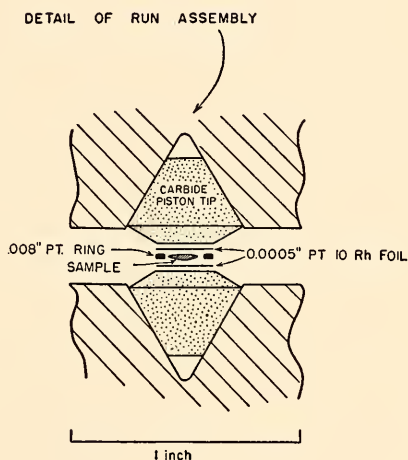
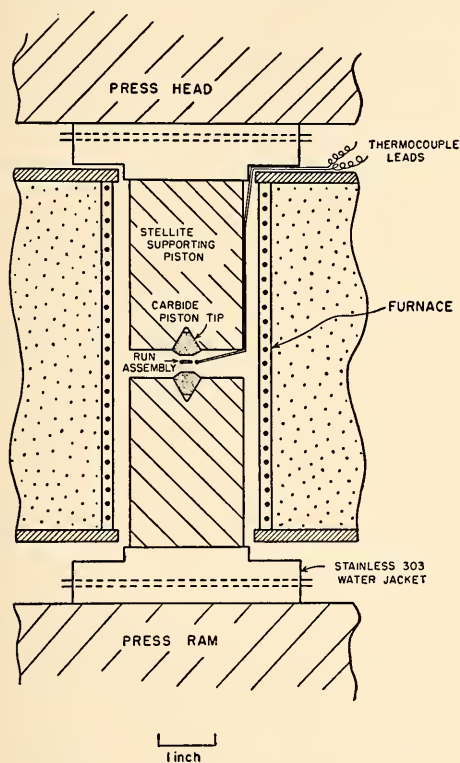


FIG. 1. Extreme pressure apparatus

ures, it has proved impossible to attain equilibrium. By the methods of thermodynamics, such results can be extrapolated into the lower pressure range which is of interest to the petrologist.

The extreme pressure apparatus in use at the Geophysical Laboratory is similar in principle to that used by Bridgman in measuring the electrical resistance of a large number of elements and alloys to 100,000 bars. Recently, Griggs and Kennedy, at the University of California, have

A sketch of the apparatus is presented in figure 1. The run, consisting of about 4 mg of sample moistened with water, is placed in a small capsule made from a platinum ring and two disks of platinum-10 rhodium foil. The capsule and sample are then compressed between the faces of pistons ground from sintered carbide. The piston faces bite on the platinum ring, making a seal which helps to prevent escape of the water. The carbide pistons are supported by being forced into cylindrical



blocks of Stellite 25. Pressure is applied by placing the piston-and-run assembly in a press.

The reaction rates observed with this apparatus are extraordinarily fast. A run length of one-half to one hour is usually sufficient to obtain definitive results. Although the sample is minute, it is sufficient for optical and X-ray study. Despite the small sample size, coarse crystallization is sometimes obtained. Single crystals of jadeitic pyroxene 0.1 mm long have been grown in our apparatus.

The investigations that have been carried out in this Laboratory in the extreme pressure region have centered around studies of the inversion or breakdown under pressure of common rock-forming minerals. Reconnaissance runs have been made on a number of feldspars, feldspathoids, and denser minerals to 60,000 bars at 700° C. Transitions have been found to occur in nepheline, leucite, anorthite, and possibly fayalite. Nepheline and leucite pass through complex breakdowns under pressure, leading to several phases in the products. Anorthite has been reacted with water to form the dense hydrous phase lawsonite. At temperatures above the stability limit of lawsonite, anorthite passes through an anhydrous transition to as yet unknown phases. A transition is also believed to have been produced in the iron olivine fayalite.

From our reconnaissance data we have selected nepheline for detailed study. Nepheline is a fairly common mineral in deep-crystallizing plutonic rocks, and a pressure limit on its stability is therefore of interest. Moreover, its breakdown products are coarse-grained and gave hope that something could be learned of their chemistry.

A preliminary phase diagram for the breakdown of nepheline under pressure is presented in figure 2. The breakdown phases produced in this system are identified by the symbols  $N(1)$  and  $N(2)$ . Although we have considerable optical and X-ray data on these phases, the details of their chemical composition have not yet been worked out. The optical properties

of  $N(2)$  are close to those of jadeite, and it is evidently a pyroxene. Phase  $N(1)$  is new. The relative proportions of these phases in the breakdown products are of the order of 90 per cent  $N(1)$  and 10 per cent  $N(2)$ .

The breakdown of nepheline to  $N(1)$  and  $N(2)$  is sluggish enough in the vicinity of boundary *B* to require fluxing with water. We have, nevertheless, made the reaction go to completion in the absence of water at 700° C and 40 kilobars, using synthetic carnegieite (a high-temperature polymorph of nepheline) as starting material.  $N(1)$  and  $N(2)$  are therefore known to be anhydrous.

In the phase diagram in figure 2, boundary *A* has proved impossible to locate exactly. This boundary separates a field in which pure nepheline ( $\text{NaAlSi}_3\text{O}_8$ ) is stable from a field in which it breaks down to a nepheline of different composition plus  $N(2)$ . The amount of  $N(2)$  obtained in the pressure range above boundary *A* appears to increase with increasing pressure. Curve *A*, therefore, probably marks the appearance of an infinitesimal amount of  $N(2)$ . We know that curve *A* lies at a pressure lower than 15 kilobars at 700° C. We suspect that it may lie at a pressure less than 10 kilobars at this temperature. If  $N(2)$  has been correctly identified as jadeitic pyroxene, the composition of the nepheline in the pressure range above curve *A* probably lies on the join  $\text{NaAlSi}_3\text{O}_8$ — $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3$ .

Boundary *B* divides the fields nepheline +  $N(2)$  and  $N(1) + N(2)$ . We have traced boundary *B* over a pressure range of 10 to 35 kilobars. The curvature of boundary *B* suggests that phases on one or both sides of the boundary are shifting composition. A shift in composition of at least the nepheline above curve *B* is also indicated by the increased yield of  $N(2)$  as the pressure is increased along curve *B*.

Curve *C* must exist, but we have no data on its location or slope other than a hydrothermal run at 425° C and 2000 bars, in



which carnegieite was recrystallized to nepheline.

We have run three natural nephelines at pressures up to 50 kilobars at 600° C and up to 20 kilobars at 800° C. One of these nephelines is from Bancroft, Ontario; the other two are from Burma. The analyses of the nephelines from Burma indicate that they are richer in  $\text{NaAlSiO}_4$  than

phase was not produced in any of the runs starting with natural nepheline. It is probable that the solution of  $\text{KAlSiO}_4$  in the natural nephelines depresses curve *B* into a temperature region below that investigated.

The results of the nepheline study suggest that jadeitic pyroxene will be a phase stable at high pressure in compositions

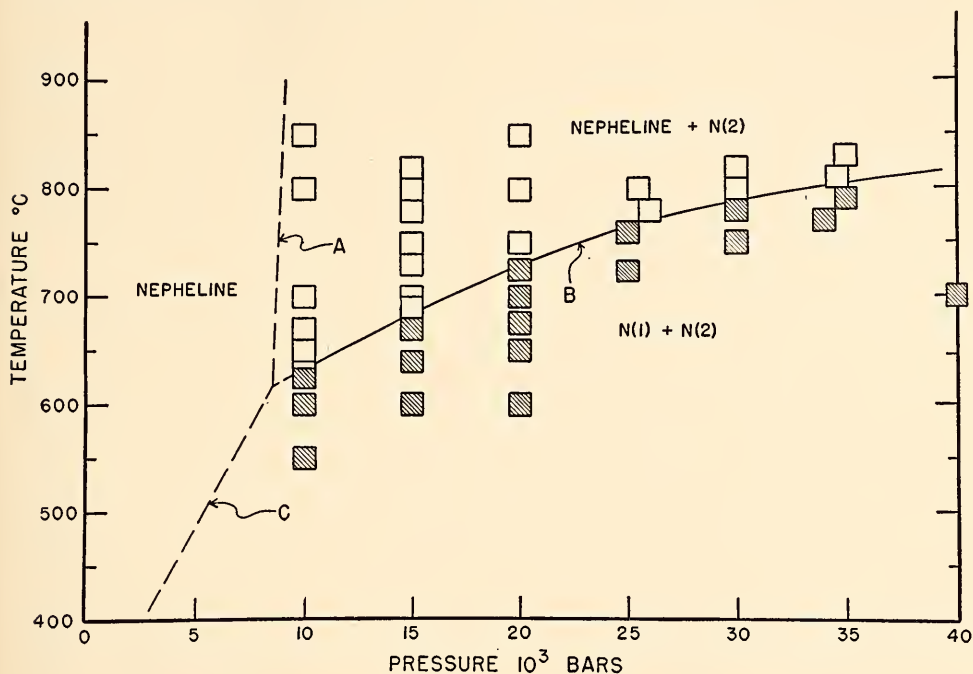


FIG. 2. Pressure-temperature diagram for the composition  $\text{NaAlSiO}_4$

other known natural nephelines. These two contain, however, nearly 15 per cent  $\text{KAlSiO}_4$  as well as excess  $\text{SiO}_2$ . All three natural nephelines were found to exsolve considerable amounts of jadeitic pyroxene at high pressure. The amount of jadeitic pyroxene produced at 20 kilobars was sufficient to be easily detected optically. In runs at 50 kilobars, its amount had increased to the point where peaks characteristic of jadeite appeared on the spectrometer patterns, i.e. an amount of the order of 10 per cent. Nevertheless, the  $\text{N}(1)$

along the entire join  $\text{NaAlSiO}_4$ — $\text{NaAlSi}_3\text{O}_8$ . Jadeite as a phase, or  $\text{NaAlSi}_2\text{O}_6$  as a component in pyroxene, is assuming increased importance as a probable host to soda at depths in the earth below the Mohorovičić discontinuity. The progressive increase with pressure in the amount of jadeitic pyroxene relative to associated less dense phases that we have observed in this system points up the possibility that phase changes in the mantle may be progressive over a large depth range.

## STABILITY OF HYDROUS IRON SILICATES

H. P. EUGSTER

In the earth's crust and on the surface a continuous adjustment of materials to the changing physical and biological environments manifests itself. A significant part of this adjustment is represented by oxidation and reduction reactions involving oxygen, iron, carbon, and many other substances which can participate in a transfer of electrons. Chemists have long recognized the importance of these phenomena and have thoroughly investigated redox systems at room temperature and atmospheric pressure. These studies cover only a small part of the conditions obtaining in the crust, however, and the geologist wishes to understand the occurrences of minerals and mineral assemblages formed under more drastic conditions. The necessary experiments are difficult to perform because, in addition to temperature and pressure, the state of oxidation of the solids must also be controlled, as well as the composition of the gas phase involved.

Although several geologically important phase diagrams for dry systems with iron as a component are now available, systematic hydrothermal investigations at high pressures had been restricted to systems which do not contain any oxidizable elements. This meant that phase relations of many important members of the chlorite, mica, amphibole, and pyroxene groups could not be investigated. In order to close this gap it was first necessary to devise a method which would allow us to study the influence of the partial pressure of oxygen (or the redox potential) on the phase relations of such silicates in a hydrothermal environment.

This has now been accomplished by employing a simple principle in conjunction with the data on the stability of iron oxides as determined by Darken and Gurry. The method used is based on the fact that for pairs of iron oxides the  $P_{O_2}$  in equilibrium with each pair is fixed for a given pressure and temperature and that it is known

over a considerable range of pressures and temperatures. The experimental design consists of a double tube arrangement with the iron oxides acting as regulators for the oxygen pressure.

An important result of this new technique is that it not only permits synthesis of several new iron silicates, but also provides the tools for a systematic investigation of reduction and oxidation reaction at high temperatures and pressures. This is of considerable importance, as not many reactions involving rock-forming mineral assemblages are completely free from changes of the metal-to-oxygen ratio in the participating solids, particularly if hydrous phases are involved.

Since it was desirable to concentrate efforts on a single test mineral and to investigate its fields of stability in terms of the three variables pressure, temperature, and partial pressure of oxygen, iron-rich biotite as represented by the end member annite,  $KFe_3AlSi_3O_{10}(OH)_2$ , was selected as the test mineral. It was chosen because many important reactions between mafic minerals involve biotites and also because recent data on the iron-free micas are available. It is clear that many conclusions based on these experiments can be generalized to include a large number of iron-bearing silicates.

The experiments were performed in the region between 300° and 900° C, between 200 and 2000 bars total pressure, and between  $10^{-12}$  and  $10^{-24}$  atm of oxygen. Much of the original experimental difficulty with hydrous systems containing iron stems from the fact that the thermal dissociation of water under these conditions is appreciable. The equilibrium constant for this reaction can be calculated from the thermochemical properties of the gases:

$$(K_P)_{T, P_{tot}} = \frac{(P_{H_2})^2 \times P_{O_2}}{(P_{H_2O})^2}.$$

For a fixed temperature and water vapor

pressure the product  $[(P_{H_2})^2 \times P_{O_2}]$  is constant. In systems that employ open or sealed platinum tubes as containers, hydrogen leaves the system continuously during the run, thereby increasing the partial oxygen pressure. Equilibrium cannot be attained in this way, because different assemblages will be stable at different partial pressures of oxygen. Therefore the  $P_{O_2}$  was held constant during the experiments by using a pair of iron oxides as buffers. The partial oxygen pressure in equilibrium with pairs of iron oxides for a given temperature and 1 atm total pressure has been determined by Darken and Gurry by varying the  $CO_2$ - $CO$  atmosphere and the temperature. Results for the reactions (a)  $Fe + O \rightleftharpoons FeO$ , (b)  $3FeO + \frac{1}{2}O_2 \rightleftharpoons Fe_3O_4$ , (c)  $3Fe_2SiO_4 + O_2 \rightleftharpoons 2Fe_3O_4 + 3SiO_2$ , and (d)  $2Fe_3O_4 + \frac{1}{2}O_2 \rightleftharpoons 3Fe_2O_3$  are presented in figure 3. The data for the curves (a), (b), (c), and (d) were recalculated in terms of the partial oxygen pressure and corrected for the change of the chemical potential of oxygen due to the total pressure on the solids. The slope of the curves (a), (b), (c), and (d) is given by the volume change  $\Delta V_F$  of the gas involved in the reactions. For all these reactions, oxygen is liberated on the left-hand side of the equation. Therefore, the  $\Delta V_F$  is strongly positive, and all the curves slope to the right. These curves form a grid of fixed and known partial oxygen pressures for any fixed temperature within the desired range.

The results of most experiments on the stability of annite as well as annite + quartz are presented in figure 3. Five independent variables are involved: composition  $X$ , temperature  $T$ , total pressure  $P_{tot}$ , water vapor pressure  $P_{H_2O}$ , and partial oxygen pressure  $P_{O_2}$ . In order to deal graphically with the system, we must keep some variables constant and project others onto the same plane. For all experiments,  $P_{H_2O} = P_{tot}$ . The stability fields are determined by varying  $T$  and  $P_{O_2}$  for a fixed total pressure. Sections for different total pressures show the influence of  $P_{tot}$  as a variable. The composi-

tion is also held constant during the runs. Two different bulk compositions are projected onto the same  $T$ - $P_{O_2}$  plane in figure 3, namely the compositions for annite and for annite + quartz.

The stability field of annite at 2000 bars total pressure is represented by the shaded area in figure 3. The boundaries are given by the lines joining the points  $Q$ ,  $A$ ,  $B$ ,  $C$ ,  $D$ ,  $E$ , and  $S$ . Point  $C$  at  $827^\circ C$  represents the temperature of the breakdown of annite to sanidine + magnetite + vapor. Point  $D$  at  $720^\circ C$  and point  $E$  at  $655^\circ C$  represent temperatures for the same reaction, but at higher partial oxygen pressures. The drop of the breakdown temperature of annite from  $827^\circ C$  at  $C$  to  $655^\circ C$  at  $E$  illustrates very clearly that the partial oxygen pressure can have a drastic influence on the stability of iron silicates. The slope of the curve  $C$ - $E$ , again, is determined only by the volume change of the gas phase during the reaction, the total pressure being held constant. The curve stands for the reaction annite + oxygen  $\rightarrow$  sanidine + magnetite + vapor. With oxygen on the left-hand side of the reaction,  $\Delta V_F$  is strongly negative and the curve slopes to the left. The drop of the partial pressure of oxygen between points  $C$  and  $E$  is one order of magnitude.

The partial oxygen pressure of point  $E$  is the highest partial oxygen pressure at which annite is still stable. Within the hematite field annite can be broken down at temperatures as low as  $400^\circ C$ . It is very likely that annite is not stable within the hematite field at any pressures and temperatures. Curve (d) was therefore taken as the upper boundary of the annite field. Point  $A$  at  $805^\circ C$  is a point for the equilibrium annite  $\rightarrow$  fayalite + leucite + O-kalsilite + vapor. The field of stability for this assemblage lies between points  $P$ ,  $A$ ,  $B$ , and  $O$ . This assemblage is equivalent to the breakdown assemblage of phlogopite, forsterite + leucite + O-kalsilite + vapor, but the univariant curve for the iron composition lies  $300^\circ$  lower.

During the experiments it was found that the vapor pressure of annite was such

that under all conditions some iron in the buffer was oxidized to wustite. It is concluded that the stability field of annite

quartz  $\rightarrow$  pyroxene + K-feldspar + water is very important for rocks of the granulite and sanidine hornfels facies as well as the

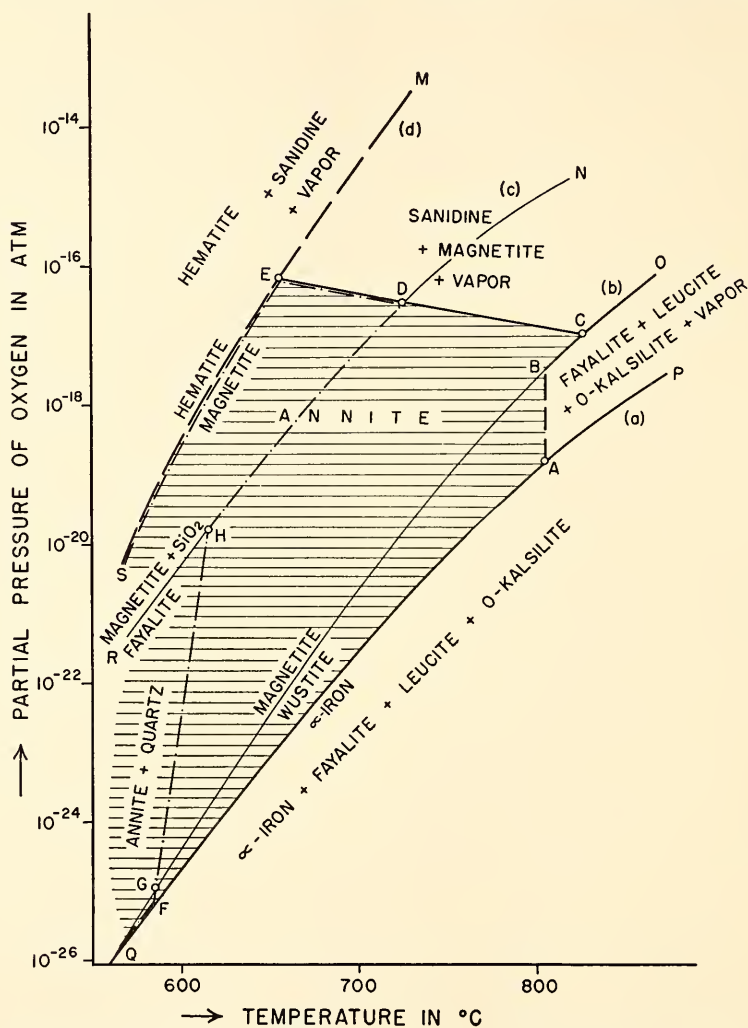


FIG. 3. Preliminary equilibrium diagram representing the stability fields of annite and annite + quartz in terms of partial pressure of oxygen and temperature. Isobaric section at a total pressure of 2000 bars. The stability field of annite is given by  $QABCDES$ ; that of annite + quartz, by  $QFGHDES$ . The breakdown assemblages are labeled for the annite composition only. Curves (a), (b), (c), and (d) are calculated from data by Darken and Gurry as explained in the text.

is terminated by the curve (a) below point A.

From a geologic viewpoint the stability of annite in the presence of free quartz is more important. The reaction biotite +

charnockites. The results of the experiments on the stability of annite in the presence of quartz are also presented in figure 3. The boundaries are now given by points Q, F, G, H, D, E, and S. At point G at



585° C, annite and quartz break down to fayalite + sanidine + vapor. For this partial oxygen pressure the stability of annite has been lowered over 200° C by the addition of quartz. Point *H* was found to be at 615° C, and the difference in the partial pressure of oxygen between points *G* and *H* is five orders of magnitude. The 30° increase from *G* to *H* can only mean that an oxygen transfer is involved in the reaction  $\text{annite} + \text{quartz} \rightarrow \text{fayalite} + \text{sanidine} + \text{vapor}$ .  $\Delta V_F$  is positive, and therefore oxygen must appear on the right-hand side, indicating that the Fe/O ratio in the synthetic annite is smaller than that in fayalite. Between points *D* and *E* the stability of annite will not be changed by the addition of quartz, as the phases on the right-hand side are still sanidine and magnetite.

In addition to the 2000-bar section, diagrams for  $P_{\text{tot}} = 1000$  bars and  $P_{\text{tot}} = 500$  bars have also been worked out. From these data it is possible to derive the shape of the three-dimensional stability area of annite with *T*,  $P_{\text{tot}}$ , and  $P_{\text{O}_2}$  as independent variables.

The experiments on the stability of annite have demonstrated very clearly that for hydrous iron silicates we cannot refer to single *P-T* curves for the breakdown reactions, unless we are able to establish that the Fe/O ratio remains constant during the reaction. Whenever a transfer of oxygen takes place during a reaction, the position of the equilibrium will be affected by the partial oxygen pressure.

If we can extrapolate the findings on the stability of annite to other hydrous ferrous silicates, we may expect to find that minerals such as greenalite, daphnite, minnesotaite, epidote, actinolite, and grunerite will be stable only over a limited range of partial oxygen pressures. In the case of annite the maximum permissible  $P_{\text{O}_2}$  for a given temperature was found to be the partial oxygen pressure of a magnetite-hematite assemblage at the same temperature. This upper limit might well apply to other ferrous silicates. Detailed field investigations from this point of view as well as more experimental studies are needed to supply the necessary information.

## THE AGE OF ROCKS AND MINERALS

(A co-operative program of the Geophysical Laboratory and the Department of Terrestrial Magnetism of the Carnegie Institution of Washington)

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The initial goal of the age group, discovery of a widely applicable means of obtaining trustworthy determinations of the age of rocks and minerals, has now been substantially achieved. Several years ago the sole method available for internal checks on reliability was a comparison of the two uranium-lead ages. Very few minerals suitable for such measurements are available, and the value of this technique is limited. A new procedure applicable to mica, a mineral of widespread occurrence,

has been developed: the comparison of the age measured by the decay of potassium<sup>40</sup> to argon<sup>40</sup> and that measured by the decay of rubidium<sup>87</sup> to strontium<sup>87</sup>. When these ages are the same within experimental error, the age of the mineral may be considered established.

### THE HALF-LIFE OF RUBIDIUM<sup>87</sup>

Rubidium<sup>87</sup> decays to strontium<sup>87</sup> with a half-life,  $T_{1/2}$ , according to the radioactive decay relation

$$T_{1/2} = \frac{0.693t}{\ln(1 + \text{Sr}^{87}/\text{Rb}^{87})}$$

where *t* is the age. Published values for the half-life obtained by counting techniques

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TABLE 1

RADIOGENIC  $\text{Sr}^{87}/\text{Rb}^{87}$  RATIOS FOR DIFFERENT MINERALS FROM THE BROWN DERBY PEGMATITE, GUNNISON COUNTY, COLORADO

Mineral	$\text{Rb}^{87}$ (ppm)	Radiogenic $\text{Sr}^{87}$ (ppm)	$\text{Sr}^{87}/\text{Rb}^{87}$
Muscovite .....	$1870 \pm 50$	$36.5 \pm 1$	0.0195
Microcline .....	$2690 \pm 100$	$48.7 \pm 1$	0.0181
Lepidolite (coarse books) .....	$5550 \pm 200$	$110 \pm 3$	0.0198
Lepidolite (medium grain) .....	$5570 \pm 200$	$121 \pm 3$	0.0210
Lepidolite (medium grain) .....	$5570 \pm 200$	$114 \pm 3$	0.0195
Lepidolite (fine grain) .....	$5890 \pm 200$	$130 \pm 3$	0.0220
Lepidolite (white) .....	$6130 \pm 200$	$118 \pm 3$	0.0192
Lepidolite (coarse) .....	$6870 \pm 200$	$134 \pm 3$	0.0195

range from  $6.3$  to  $4.3 \times 10^{10}$  yr with a single geologically deduced value lying at the high end of the range. These counting experiments are considered somewhat unreliable because of the difficulty of counting the entire energy spectrum of the emitted electrons, which has a high preponderance of low-energy beta particles.

Examination of the values obtained for the ratio of radiogenic strontium<sup>87</sup> to rubidium<sup>87</sup> for eight mineral samples from one pegmatite showed that the ratio was a constant independent of the concentration of rubidium and also independent of the structure of the minerals examined. The

results of this study are given in table 1. The constancy obtained indicates that the ratios similarly calculated for other rock units are also characteristic of the units themselves.

A reference time scale was determined, ranging from 375 million to 2700 million years ago, based on concordant U-Pb ages from six locations, as shown in the first four columns of table 2. Five of these determinations were made at the Carnegie Institution, and one has been recalculated after Holmes. These values are to be considered reliable because of the accuracy of the decay constants for the isotopes of

TABLE 2

URANIUM-LEAD AGES AND RATIOS, RADIOGENIC  $\text{Sr}^{87}/\text{Rb}^{87}$  FOR MINERALS FROM SIX PEGMATITES  
(The column  $T_{1/2}\text{Rb}^{87}$  is calculated using the  $\text{U}^{235}\text{-Pb}^{207}$  age.)

Pegmatite location	U-Pb ages ( $\times 10^{-6}$ yr)			Rb-Sr data		$T_{1/2}\text{Rb}^{87}$ ( $10^{10}$ yr)
	Mineral	$\text{U}^{238}\text{-Pb}^{206}$	$\text{U}^{235}\text{-Pb}^{207}$	Mineral	Radiogenic $\text{Sr}^{87}/\text{Rb}^{87}$	
Bikita Quarry, S. Rhodesia. . .	Monazite*	$2640 \pm 100$	$2680 \pm 100$	Lepidolite	$0.0380 \pm 0.0010$	5.0
Viking Lake pegmatite, Sask. .	Uraninite	$1790 \pm 50$	$1830 \pm 50$	Biotite	$0.0270 \pm 0.0010$	4.8
Bob Ingersoll Mine, Keystone, S. D. ....	Uraninite	$1580 \pm 30$	$1600 \pm 30$	Lepidolite	$0.0239 \pm 0.001$	4.8
				Muscovite	$0.0244 \pm 0.001$	
				Microcline	$0.0224 \pm 0.001$	
Cardiff Uranium Mine, Cardiff Township, Ont. ....	Uraninite	$1020 \pm 20$	$1020 \pm 20$	Biotite	$0.0140 \pm 0.0007$	5.1
Fission Mine, Wilberforce, Ont.	Uraninite	$1040 \pm 20$	$1050 \pm 20$	Biotite	$0.0140 \pm 0.0007$	5.2
Chestnut Flat Mine, Spruce Pine, N. C. ....	Uraninite	$375 \pm 10$	$380 \pm 10$	Muscovite	$0.00515 \pm 0.0002$	5.0
				Microcline	$0.00535 \pm 0.0002$	

\* Recalculated after A. Holmes, *Nature*, vol. 173, p. 612 (1954).

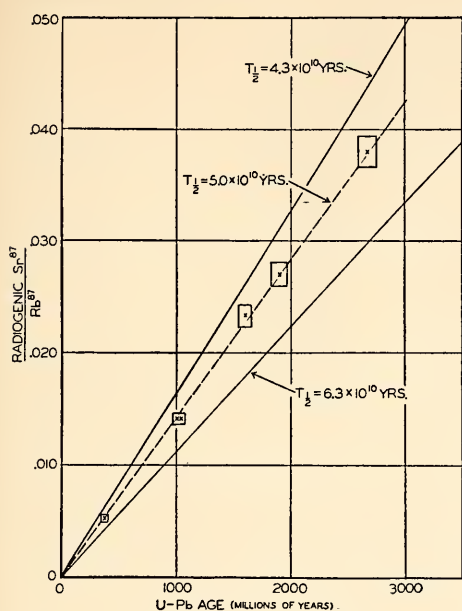


FIG. 4. Ratios (radiogenic  $\text{Sr}^{87}$ )/ $\text{Rb}^{87}$  for minerals from rock units with concordant U-Pb ages versus the U-Pb ages.

uranium and the concordance of the ages shown by these minerals. Values of the rubidium half-life, calculated from the ratio of radiogenic strontium<sup>87</sup> to rubidium<sup>87</sup> from rubidium-containing minerals from the same localities, are shown in the last three columns of table 2. The average of the values thus obtained,  $5.0 \pm 0.2 \times 10^{10}$  yr, lies within the range of values found by direct counting experiments, shown by the solid lines in figure 4. This geologically

determined value has very recently been confirmed by direct counting experiments, according to a letter from E. Huster, who in 1954 published the lower value shown in the figure.

The radioactive decay of potassium<sup>40</sup> is more complicated than that of rubidium<sup>87</sup> in that the parent element decays by electron capture to argon<sup>40</sup> and by beta emission to calcium<sup>40</sup>, with corresponding decay constants  $\lambda_e$  and  $\lambda_\beta$ . The K-A age for young minerals depends almost linearly on  $\lambda_e$  (Wetherill *et al.*, 1956); thus comparison of the  $\text{A}^{40}/\text{K}^{40}$  ratio of a young mineral with a concordant U-Pb age will permit the determination of  $\lambda_e$  with very little regard to  $\lambda_\beta$ . This calculation had been made earlier by others, who used potassium feldspar as the index mineral. Comparisons of feldspars with micas from the same rock showed that mica consistently gave higher ratios of  $\text{A}^{40}/\text{K}^{40}$ —an indication that the feldspars probably had lost some argon during geologic time. Table 3 gives this comparison for seven pairs of associated minerals.

Comparisons were made of eight samples or sample pairs from different localities, as shown in table 4, to evaluate  $\lambda_e$ . The value of  $\lambda_\beta$  is that calculated by P. M. Endt and J. C. Kluyver (1954). The K-A age values are relatively insensitive to this value. The value of  $\lambda_e$  inferred from the U-Pb ages is not significantly different from the average of ten direct counting experiments from

TABLE 3

COMPARISON OF  $\text{A}^{40}/\text{K}^{40}$  RATIOS OF FELDSPARS AND MICAS FROM THE SAME ROCK

Location	Rock type	$\text{A}^{40}/\text{K}^{40}$	
		Feldspar	Mica
Quartz Creek, Colo.....	Granite	0.0725	0.107
Uncompahgre, Colo. ....	Granite	0.0709	0.109
Cape Town, S. Africa.....	Granite	0.0105	0.0337
Ohio City, Colo.....	Pegmatite	0.0590	0.0974
Black Hills, S. D.....	Pegmatite	0.0862	0.118
Jakkalswater, S. Africa.....	Pegmatite	0.0359	0.0695
Wilberforce, Ont. ....	Pegmatite	0.0614	0.0695

TABLE 4  
COMPARISON OF K-A AND U-Pb AGES  
( $\lambda_e = 0.557 \times 10^{-10} \text{ yr}^{-1}$ ;  $\lambda_\beta = 0.472 \times 10^{-9} \text{ yr}^{-1}$ )

Sample	$A^{40}/K^{40}$	K-A age (million years)	U-Pb age (million years)
Spruce Pine, N. C.....	0.0213	349	375 $\pm$ 10
Parry Sound, Ont.....	0.0705	970	994
Cardiff Township, Ont.....	0.0729	1000 $\pm$ 50	1020 $\pm$ 20
Wilberforce, Ont. ....	0.0695	960 $\pm$ 50	1030 $\pm$ 30
Keystone, S. D.....	0.140	1600 $\pm$ 80	1600 $\pm$ 30
Keystone, S. D.....	0.119	1430 $\pm$ 70	1600 $\pm$ 30
Viking Lake, Sask.....	0.175	1850 $\pm$ 80	1890 $\pm$ 40
Bikita, S. Rhodesia.....	0.300	2550 $\pm$ 150	2650 $\pm$ 100*

\* A. Holmes, *Nature*, vol. 173, p. 612 (1954).

the literature; the individual published values have a spread of about 25 per cent, however.

#### APPLICATION OF NEW VALUES OF DECAY CONSTANTS

The determination of the decay constants for rubidium<sup>87</sup> and potassium<sup>40</sup> by comparison of the ages of micas with good U-Pb ages justifies considerable confidence in the ages determined for micas from localities where good U-Pb ages are not available. Although the possibility does exist of a small degree of loss of argon in a few cases, the effect is slight. In the work reported previously, the ages were based on arbitrary constants, for lack of better information. It is now possible to recalculate the values to give reliable ages. A summary table (table 5) has been prepared which serves to collect all the ages measured since the beginning of the program. The results in the table, computed using the new constants, supersede all previous values. To simplify the table, errors have not been stated but are less than  $\pm 5$  per cent in practically all cases.

The relative agreement between K-A ages and Rb-Sr ages of micas calculated with the geologically determined decay constants is emphasized by the histogram in figure 5. Agreement between these two

ages obtained for a single sample would seem to be an even better criterion for evaluating the true age than agreement between the two uranium-lead ages, because rubidium and strontium decay to chemically different daughters; thus any changes affecting the mineral might be expected to affect the two parent-daughter ratios differently. In the case of the uranium-lead system, it is mainly the difference in half-lives of the two uranium parent isotopes which is depended on in using concordant ages as a measure of age.

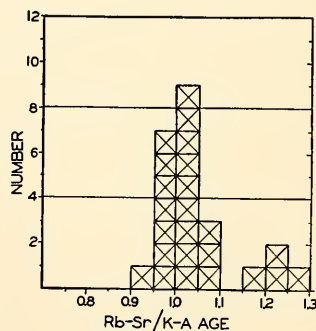


FIG. 5. Agreement between Rb-Sr ages and K-A ages of micas.

#### DISCORDANT URANIUM-LEAD AND THORIUM-LEAD AGES

The lack of agreement frequently found among the U-Pb and Th-Pb ages lies out-



TABLE 5  
SUMMARY OF MINERAL AGE DETERMINATIONS 1953-1956  
(Millions of years)

Locality	Mineral	Occurrence	K-A	Rb-Sr	U <sup>238</sup> - Pb <sup>206</sup>	U <sup>235</sup> - Pb <sup>207</sup>	Pb <sup>207</sup> - Pb <sup>206</sup>	Th <sup>232</sup> - Pb <sup>208</sup>
Pala, Calif. ....	lep	p	...	106	...	...	...	...
Conway, N. H., Redstone.....	bio	g	144	185	...	...	...	...
	zir	g	...	...	193	...	...	210
Spruce Pine, N. C., Chestnut Flat Mine .....	ur	p	...	...	370	375	...	...
	mu	p	355	375	...	...	...	...
	mi	p	...	385	...	...	...	...
Alto Ligonha, Mozambique.....	lep	p	...	435	...	...	...	...
Dogon Daji, Nigeria.....	lep	p	...	475	...	...	...	...
Sahatany, Ambositra, Madagascar .....	lep	p	...	535	...	...	...	...
Cape Town, S. Africa.....	bio	g	530	660	...	...	...	...
	zir	g	...	...	330	356	530	238
Ceylon (gem) .....	zir	p	...	...	540	544	555	538
Okongova Ost, Karabib, SW. Africa .....	mu	p	...	550	...	...	...	...
	lep	p	...	471	...	...	...	...
	lep	p	...	545	...	...	...	...
Ruhuma, Kigezi, Uganda.....	lep	p	...	800	...	...	...	...
OP 19, Goodhouse District, Cape Province, S. Africa.....	mon	p	...	...	930	915	880	900
Muika, Belgian Congo.....	lep	p	...	935	...	...	...	...
	lep	p	...	950	...	...	...	...
Jakkalswater, Namaqualand.....	lep	p	970	960	...	...	...	...
Houtenbek, near Pretoria, S. Africa .....	mon	p	...	...	1400	1230	930	940
Kinderzitt, Umeis, Nama- qualand .....	lep	p	...	985	...	...	...	...
Hybla, Ont. ....	cyr	p	...	...	1350	1190	900	435
Wilberforce, Ont., Fission Mine.	ur	p	...	...	1040	1050	1070	1010
	bio	p	975	1000	...	...	...	...
Bancroft, Ont. ....	bio	p	900	990	...	...	...	...
Cardiff Township, Ont., Cardiff Mine .....	ur	p	...	...	1020	1020	1020	955
	bio	p	1010	1030	...	...	...	...
	zir	p	...	...	900	930	1000	990
Pikes Peak, Colo.: Near Manitou Springs.....	bio	g	1030	1020	...	...	...	...
	zir	g	...	...	624	707	980	313
Top of peak.....	bio	g	1100	1080	...	...	...	...
Llano Co., Texas, Petrick Quarry .....	bio	g	1090	1100	...	...	...	...
	zir	g	...	...	950	990	1070	890
Steenkampskraal, Cape Prov- ince, S. Africa .....	mon	p	...	...	1080	...	...	990
Tory Hill, Ont., Haliburton Co.	zir	g	...	...	1030	1050	1090	390
	zir	syen	...	...	940	960	1015	...
	sph	g	...	...	910	...	...	...
	sph	g	...	...	...	...	1090	450
Natural Bridge, N. Y.....	zir	c	...	...	1025	1065	1140	...

(Continued on next page)

TABLE 5—*Continued*

Locality	Mineral	Occurrence	K-A	Rb-Sr	U <sup>238</sup> .. Pb <sup>206</sup>	U <sup>235</sup> .. Pb <sup>207</sup>	Pb <sup>207</sup> .. Pb <sup>206</sup>	Th <sup>232</sup> .. Pb <sup>208</sup>
Dixon, N. M., Harding Mine	mu	p	...	1320	...	...	...	...
	mu	p	...	1290	...	...	...	...
	lt m'lte	p	...	...	970	960	940	...
	dk m'lte	p	...	...	920	1000	1130	...
Gunnison Co., Colo., Brown Derby Mine	m'lte	p	...	...	915	1055	1350	...
	cb-ta	p	...	...	1520	1470	1390	...
	mon	p	...	...	1590	1420	1170	995
	lep	p	1360(5)	1410(7)	...	...	...	...
	mi	p	830	1290	...	...	...	...
Gunnison Co., Colo., Quartz Creek	bio	g	1320	1310	...	...	...	...
	zir	g	...	...	925	1130	1540	530
	zir	g	...	...	...	...	1700	...
Mesa Co., Colo., Uncompahgre	bio-xeno	g	1360	1370	3180	2065	1640	1100
Sherman, Wyo.	bio	g	1410	1420	...	...	...	...
Bagdad, Ariz.	mu	g	1410	1390	...	...	...	...
	zir	g	...	...	630	770	1210	270
	lep	p	1410	1500	...	...	...	...
Black Hills, S. D., Ingersoll Mine, Keystone	lep	p	1430	1690	...	...	...	...
	ur	p	...	...	1580	1600	1630	1440
	mu	p	1600	1730	...	...	...	...
	mi	p	1120	1590	...	...	...	...
Varuträsk, Sweden	lep	p	...	1770	...	...	...	...
Nampeyo Hill, Uganda	mu	p	...	1840	...	...	...	...
Viking Lake, Sask.	ur	p	...	...	1790	1830	1870	1640
	bio	p	1850	1970	...	...	...	...
Lunya, near Nagoje, Uganda	mu	p	...	1870	...	...	...	...
M'bale, Uganda	mu	p	...	1910	...	...	...	...
	mu	p	...	1830	...	...	...	...
Bonneville, Wyo., Whippet No. 1	lep	p	2360	2470	...	...	...	...
Forfar, Shangangwe, S. Rhodesia	lep	p	...	2490	...	...	...	...
Hombolo, Tanganyika	lep	p	...	2500	...	...	...	...
Bikita Quarry, S. Rhodesia	lep	p	2550	2680	...	...	...	...
Winnipeg River, SE, Manitoba, Silver Leaf Mine	lep	p	...	2640	...	...	...	...
Pope's Claim No. 1, Salisbury, S. Rhodesia	lep	p	...	2810	...	...	...	...
Willie 481, Letaba, Transvaal	lep	p	...	2810	...	...	...	...
Grosmont, W. Australia	lep	p	2810	2640	...	...	...	...
	mi	p	2750	2740	...	...	...	...
	lep from mi	p	2780	2800	...	...	...	...
Catlin Creek, W. Australia	mu	p	2830	2810	...	...	...	...
	mi	p	2170	2710	...	...	...	...
Wodgina, W. Australia	mu	p	2400	2950	...	...	...	...
	mi	p	2190	2710	...	...	...	...
Londonderry, W. Australia	mu	p	2720	3190	...	...	...	...
	mi	p	2200	2760	...	...	...	...
Kubuta, S. Swaziland	lep	p	...	3200	...	...	...	...

bio, biotite; c, contact; cb, columbite; cyr, cyrtolite; g, granite; lep, lepidolite; mi, microcline; m'lte, microcline; mon, monazite; mu, muscovite; p, pegmatite; sph, sphene; syen, syenite; ta, tantalite; ur, uraninite; xeno, xenotime; zir, zircon.

side the limits of the analytical errors. Some information about the reasons for these discrepancies has been acquired by analyses of the material obtained from the minerals by mild acid washes. Results to date suggest that low Th-Pb ages reflect loss of both thorium and lead from a mineral, with the loss of  $\text{Pb}^{208}$  predominating. High  $\text{U}^{238}\text{-Pb}^{206}$  ages result from loss of both  $\text{U}^{238}$  and  $\text{Pb}^{206}$  from a mineral, with the loss of uranium predominating. Low Th-Pb ages are commonly encountered in minerals such as uraninite and zircon, and high U-Pb ages are found for many monazites. The factors governing the loss of uranium, thorium, and lead have not yet been established. This work has been published by Tilton (1956).

A theoretical analysis of discordant uranium ages has been made by Wetherill (1956), who has shown how the discordance may be interpreted to evaluate the geochemical history of a suite of minerals in terms of the time when gains or losses of parent and daughter may have taken place.

The discordant ages often obtained from measurements of U-Pb in zircons was the subject of a further study, results of which are summarized in table 6. In some zircons

comparatively large amounts of primary lead were present; these were also the specimens giving discordant ages. One is tempted to suggest that this primary lead indicates a process in which the original zircon mineral was invaded not only by lead but also by uranium and thorium, to account for the values observed.

#### GEOCHEMISTRY OF LEAD AND ITS PARENTS

Two years ago the isotopic composition of lead in two olivine nodules was reported. It was pointed out at that time that the U/Pb ratio in these nodules is such that the isotopic composition of their lead would not have changed appreciably in the past four to five billion years if they had always contained their present amounts of uranium and lead. It was found that the lead in the nodules is a "modern" lead, which until recent times was in an environment that had a U/Pb ratio some twenty times higher than that of the nodules themselves.

In the past year these measurements were extended to include a massive dunite from Twin Sisters, Washington. The dunite gave results similar in every respect to those obtained on the nodules.

TABLE 6  
LEAD CONTENT AND LEAD AGES OF ZIRCONS

Locality	Primary lead (ppm)	Total lead (ppm)	AGE (MILLION YEARS)			
			$\text{U}^{238}\text{-Pb}^{206}$	$\text{U}^{235}\text{-Pb}^{207}$	$\text{Pb}^{207}\text{-Pb}^{206}$	$\text{Th}^{232}\text{-Pb}^{208}$
Cape Town, S. Africa.....	14	66.5	330	356	530	238
Ceylon (gem) .....	$\leq 1.7$	127	540	544	555	538
Cardiff Township, Ont., Cardiff Mine .....	$\leq 6$	485	900	930	1000	990
Pikes Peak, Colo., Manitou Springs..	3	33.3	624	707	980	313
Llano Co., Texas, Petrick Quarry..	$\leq 0.6$	53.8	950	990	1070	890
Tory Hill, Ont., Haliburton Co....	(g) $\leq 5.5$	461	1030	1050	1090	390
	(syc) $\leq 1.5$	123	940	960	1015	...
Natural Bridge, N. Y.....	$\leq 1.6$	127	1025	1065	1140	...
Gunnison Co., Colo., Quartz Creek..	54	210	925	1130	1540	530
Bagdad, Ariz. ....	40	338	630	770	1210	271
Conway, N. H.* .....	...	...	193	...	...	210

g, granite; syc, syenite.

\* Preliminary result.

Since the leads require higher U/Pb ratios than those now found in the ultramafics, a differentiation of some sort is required. So far the lead isotope work has not revealed a possible parent material for the ultramafics. In one specimen, from Hualalai, Hawaii, the isotopic composition of lead in both the olivine nodule and the accompanying basalt has been determined. The nodule and basalt leads have small but significant differences in isotopic composition which rule out the basalt itself as

a source for the olivine nodules. Table 7 gives the uranium, thorium, and lead content as well as the isotopic composition of lead from these rocks.

The measurement of the isotopic composition of lead obtained from ultrabasic rocks, volcanic nodules, and basalt flows gives promise that such diverse phenomena as magmatic differentiation, partial fusion of deep-seated rocks, heat flow beneath the ocean, and the origin of meteorites may be deductively investigated.

TABLE 7

URANIUM, THORIUM, AND LEAD CONTENT AND ISOTOPIC COMPOSITION OF LEAD FROM BASALTS AND ULTRAMAFIC ROCKS

Source	Sample	PARTS PER MILLION			ATOM RATIOS		
		U	Th	Pb	Pb <sup>206</sup> / Pb <sup>204</sup>	Pb <sup>207</sup> / Pb <sup>204</sup>	Pb <sup>208</sup> / Pb <sup>204</sup>
Dreiser Weiher, Germany							
Nodule .....	Diopside	...	...	2	19.43	15.30	37.86
San Bernardino, Calif.							
Nodule .....	Diopside	0.007	...	2	19.16	15.46	37.78
Twin Sisters, Wash.							
Dunite .....	Dunite minus spinel	0.016	0.05	1.6	19.17	15.61	38.15
Hualalai, Hawaii							
Nodule* .....	Nodule minus spinel	0.005	...	1	19.29	15.45	37.95
Hualalai, Hawaii							
Basalt .....	Basalt minus spinel	0.50	1.60	2.6	18.62	15.61	38.36

\* Analysis: C. C. Patterson, California Institute of Technology.

## PRIMITIVE MAGMAS

### SOURCES OF MAGMA

The search for a primitive source from which all magmas might be derived is one of the most engrossing petrological problems. Irrespective of one's beliefs as to the formation of the earth—by crystallization of a hot magma or by cold accretion of extraterrestrial material—one is faced with the problem of the source of the present observable molten rock-forming materials, as well as their ancient cooled equivalents. Researches have suggested that igneous rocks of even the most diverse composition are related by virtue of a line or lines of primarily thermal descent from a common source.

Field investigators recognize two principal lines of descent: the alkali olivine-basalt→mugearite→trachyte→pantellerite series, and the tholeiite basalt→tholeiitic andesite→dacite→rhyolite series. Some believe that both lines of descent may stem from an alkali olivine-basalt; others present arguments indicating that the tholeiite basalt is the source magma of both; still others prefer the notion of two or more separate magmas, each producing its products independent of the other; finally, there are those who have proposed a more basic magma from which the above-named magma series are derived. To date, the indications are that all lines of descent are



to "petrogeny's residua system," in which the rhyolites and granites are found. Unfortunately, none of the proposed primitive magmas is observed in volumes sufficient to produce the volume of rhyolites and granites now exposed. Of particular significance to the study of the nature of the source magma is the diopside—orthoclase—ferrosilite—silica system as outlined by Osborn and Tait (1952). The compositions in this system approach those of iron-free magmas. The results of this study suggest that two lines of descent are in fact possible, each having features characteristic of one of the two proposed magma series.

The most significant differences between the two principal magmas involve the pyroxenes. The tholeiite basalt series shows the reaction relation of olivine with liquid to yield pyroxene, whereas the alkali basalt series does not. The tholeiite basalt has normative hypersthene; the alkali basalt has little or none of this constituent relative to diopside in the norm. It is clear that laboratory investigation of the pyroxenes holds one of the keys to the primitive basalt problem. The relations of the pyroxene solvus and solidus surfaces probably play a role in the basalts similar to that of the relations of the feldspar solvus and solidus in the granites.

#### NATURAL THOLEIITE BASALT—WATER SYSTEM

H. S. YODER, JR., AND C. E. TILLEY

The laboratory studies on systems involving the principal components of magma will eventually lead to an understanding of the common magma types. In the hope of gaining some insight into the general relations in advance of the detailed results, a direct but less rigorous approach to the problem has been attempted. Since a rock may be treated as a single bulk composition in a multicomponent system, the course of crystallization of this composition may be followed in the usual way by the quenching method. Toward this end, samples of representative basalts are being subjected

to various pressures and temperatures. Preliminary results on a tholeiite basalt from the 1921 flow of Kilauea, Hawaii (table 8) are presented in figure 6.

As the figure shows, at atmospheric pressure and temperatures above 1235° C the basalt is all liquid (with the exception of a few chrome spinels) and may be quenched to glass,  $n=1.608$ . The temperature of the magma at Kilauea has been measured at 1185° and 1200° C, and the index of refraction of a natural glass was 1.605. The first silicate crystals, olivine, appear at 1235° C. These are joined at 1190° C by pyroxene, and at 1170° C by feldspar. The appearance of the three principal minerals of a basalt within a narrow range of temperature is in accord with the observations at Kilauea. At 1090° C the tholeiite basalt is almost completely crystalline. Magnetite forms early in the melt, but its first appearance has not been located with certainty under equilibrium conditions.

These relations change drastically and discontinuously with water pressure. At 5000 bars, for instance, the basalt is all liquid (with the exception of some magnetite) above 1125° C. It was not possible, however, to quench this liquid to a glass under the experimental setup employed. In place of the glass that would result from a successful quench, the charge consisted almost wholly of an amphibole, with small amounts of magnetite and some glass. The physical appearance of the charge and the radial growth of the fibrous amphibole clearly indicated that this phase was a quench product. The amphibole was yellow to pale green, and had an extinction angle of about 32°. This "quench" amphibole could be easily distinguished from the amphibole described below obtained under equilibrium conditions. The presence of "quench" amphibole in a run was interpreted to represent liquid at the temperature and pressure of the run, and will be so regarded in this discussion. It is of interest to note that with the addition of the necessary water the analysis of the

TABLE 8  
OLIVINE BASALT, 1921 LAVA, KILAUEA CALDERA, HAWAII

1			2		3	
SiO <sub>2</sub> ....	49.16		P .....	0.017	0.017	
Al <sub>2</sub> O <sub>3</sub> ...	13.33	<i>Norm</i>	Si .....	6.847	6.945	8.00
Fe <sub>2</sub> O <sub>3</sub> ...	1.31	Or .....	Al .....	1.136	1.038	
FeO .....	9.71	Ab .....	Al .....	1.037	1.260	
MnO .....	0.16	An .....	Cr .....	0.017	0.017	
MgO .....	10.41	Di .....	Fe .....	0.137	0.136	
CaO .....	10.93	Hy .....	Fe''' .....	1.130	1.183	4.90
Na <sub>2</sub> O ...	2.15	Ol .....	Mn'' .....	0.017	0.017	
K <sub>2</sub> O ...	0.51	Mt .....	Mg .....	2.173	2.290	
H <sub>2</sub> O <sup>+</sup> ...	0.04	Il .....	Ti .....	0.240	....	
H <sub>2</sub> O <sup>-</sup> ...	0.05	Ap .....	Ca .....	1.626	1.464	
P <sub>2</sub> O <sub>5</sub> ...	0.16	H <sub>2</sub> O ± ..	Na .....	0.589	0.613	2.18
TiO <sub>2</sub> ...	2.29	.....	K .....	0.086	0.102	
Cr <sub>2</sub> O <sub>3</sub> ...	0.09	.....	OH ....	2.037	2.145	2.14
	100.30	100.25				

1. Analysis of lava no. 57364 (Cambridge University Collection) by J. H. Scoon, and norm.

2. Recalculation of 1 to amphibole formula after adding requisite water.

3. Recalculation of 1 to amphibole formula after removal of TiO<sub>2</sub>, SiO<sub>2</sub>, and CaO as sphene and addition of requisite water.

Kilauea basalt can be converted to that of an amphibole common in some garnet amphibolites (see table 8). Under 5000 bars water pressure at 750° C the charge was converted to an assemblage built of amphi-

bole, plagioclase, sphene, and magnetite with traces of glass. This amphibole is light to dark green, and has an extinction angle of about 20°. At this and lower temperatures the charge has many of the characteristics of a natural amphibolite. With successively higher temperatures the sequence of changes at the same water pressure was as follows: 815° C, plagioclase out (here the assemblage resembles a hornblende with sphene; see table 8); 875° C, sphene out; 925° C, pyroxene in; 975° C, amphibole out, olivine in; 1090° C, pyroxene out; 1125° C, olivine out (liquidus).

The curves in figure 6 outline in a general way the stability fields of the dominant phases. The shapes of the curves are based in part on experience gained from less complex systems and are not to be regarded as complete or final. These few preliminary experiments, although of great value in outlining the general relations, must be interpreted with extreme caution. The problems arising from investigation of a multicomponent system, i.e. a natural rock, are many. For example, the state of oxidation of iron was not controlled and is known to vary from run to run. In addition, all the major constituents are solid

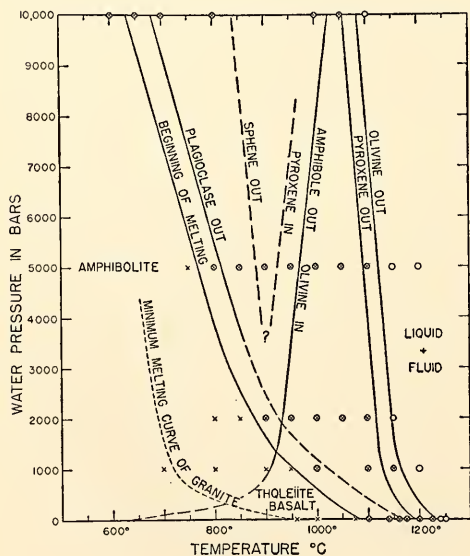


FIG. 6. Projection of the natural tholeiite basalt—water system based on reconnaissance runs on the 1921 lava of Kilauea, Hawaii. Limitations of data are noted in the text.

solutions, and the variations in their composition have not been carefully studied. These variations are of great importance as a record of the course of crystallization. One minor phase, rutile, which did not appear in the pressure sections described, forms in the low-pressure region, and its relationship to sphene has not been established. Although equilibrium was readily obtained at the higher pressures and temperatures, runs of a month's duration were not sufficient to reach equilibrium at the lowest temperatures and pressures investigated.

In spite of these difficulties one may draw some conclusions with regard to natural rocks. The magmas that crystallize to tholeiite basalt on or near the surface of the earth, or to gabbro at depth, must have had a very low water content at the time of crystallization. If water had been present and retained, the magma presumably would have crystallized as hornblende or amphibolite. Similarly, tholeiite basalt magma which has crystallized and later been exposed to a hydrothermal environ-

ment would be converted to amphibolite. The relation of the beginning-of-melting curve of amphibolite and tholeiite basalt to the minimum-melting curve of granite is readily observed in figure 6. It is significant that these two curves are only 150° to 200° C apart. The principal difference in the melting relations of granite and tholeiite basalt, however, is in the magnitude of their melting intervals. Since the compositions of most granites lie in or near the low-temperature trough of the "granite" system, much liquid is produced with a small rise in temperature above that of the minimum. On the other hand, an equivalent temperature increase above the temperature at which melting begins for tholeiite basalt results in only a small amount of liquid. It is presumed that sediments approaching the compositions of granites and basalts will also behave in this manner.

Other representative basalts to be studied include an alkali olivine-basalt from Mauna Kea, Hawaii, and a high-alumina basalt from the Medicine Lake Highlands of California.

## PALEOBIOCHEMISTRY: INORGANIC SYNTHESIS OF AMINO ACIDS

P. H. ABELSON

The origin of life is a problem that for many years has attracted the attention of eminent scientists. For the most part their efforts have been devoted to speculation regarding possible mechanisms of formation of successively more complex molecular assemblages. There has been little experimental investigation or attempt to devise hypotheses which might lead to predictions that could be tested.

A major exception is the work of Stanley Miller (1955), who has studied the effects of electrical discharges in a hypothetical primitive atmosphere consisting of methane, ammonia, and steam. He has succeeded in synthesizing and identifying substantial amounts of amino acids. The specific composition of "primitive gases" employed was chosen on the basis of a suggestion by H. C. Urey that the earth's

original atmosphere was highly reducing and consisted principally of methane and ammonia.

There is, however, disagreement concerning the composition of the early atmosphere. W. W. Rubey sets forth the view that carbon dioxide and nitrogen were the principal gases. He has assembled a rather impressive mass of geological evidence which indicates that the earth's atmosphere and oceans were formed from gases issuing from the interior of the earth.

The volatiles associated with volcanoes today include H<sub>2</sub>O, CO<sub>2</sub>, N<sub>2</sub>, CO, HCl, H<sub>2</sub>S, SO<sub>2</sub>, and SO<sub>3</sub>. Following Rubey, let us consider the fate of such gases in contact with a primitive, alkaline, reducing crust. Water vapor would condense, forming shallow pools. The acidic gases would dissolve in water and react with the alka-



line rocks. Gases such as  $\text{CO}_2$ ,  $\text{H}_2\text{S}$ , and  $\text{SO}_2$  would be distributed between atmosphere and water. The primitive air then would consist principally of  $\text{N}_2$ ,  $\text{H}_2\text{O}$ ,  $\text{CO}_2$ , and  $\text{CO}$ .

In view of the conflicting Urey-Rubey viewpoints it seemed desirable to investigate amino acid synthesis in "primitive atmospheres" other than methane-ammonia and approaching more closely that advocated by Rubey.

First, it was necessary to consider some of the effects of sunlight and radiation on a  $\text{CO}_2$ ,  $\text{CO}$ ,  $\text{N}_2$ ,  $\text{H}_2\text{O}$  atmosphere. It has been pointed out that, in the absence of sizable amounts of ozone in the atmosphere (formed from free oxygen), large amounts of short-wavelength ultraviolet light would penetrate to the surface of the earth.

Let us examine qualitatively some photoreactions which would occur:  $\text{H}_2\text{O} + \nu \rightarrow \text{H} + \text{OH}$ . Both  $\text{H}$  and  $\text{OH}$  are very reactive. They may recombine, or the following reactions, among others, may occur:  $\text{H} + \text{H} \rightarrow \text{H}_2$ ,  $\text{OH} + \text{OH} \rightarrow \text{H}_2\text{O}_2$ ,  $\text{OH} + \text{H}_2\text{O} \rightarrow \text{H}_2\text{O}_2 + \text{H}$ . Hydrogen peroxide would in part be redissociated into its original components. That formed in water at the surface of the earth, however, would react with ferrous iron and other reducing agents. In addition, some of the peroxide formed in the atmosphere would be brought to earth by rain, where it would likewise be destroyed. The result of this series of events would leave the atmosphere enriched in hydrogen and more reducing.

Consider another series of reactions:  $\text{CO}_2 + \nu \rightarrow \text{CO} + \text{O}$ ,  $\text{O} + \text{O} \rightarrow \text{O}_2$ ,  $\text{O}_2 + \text{N}_2 \rightarrow 2\text{NO}$ . Nitric oxide dissolves in water to form acid, which on reacting with alkali present in surface waters would be converted into a soluble salt. The net effect would be to remove oxygen from the atmosphere. Carbon monoxide could also participate in a series of reactions. The gas is soluble in alkaline solutions, in which it is converted to formate. This compound is known to be destroyed by ultraviolet light, even by quanta of relatively long

wavelengths, to yield carbonate plus free hydrogen.

The above reactions are members of a series involving  $\text{CO}_2$ ,  $\text{CO}$ ,  $\text{N}_2$ , and  $\text{H}_2\text{O}$  having as an end result the oxidation of ferrous iron or the neutralization of alkali and the creation of a more reducing atmosphere.

Since the Miller experiments aroused widespread interest, equipment similar to his was employed, as shown in figure 7.

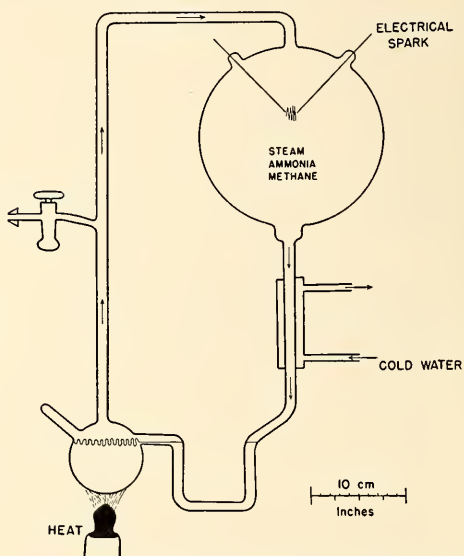


FIG. 7. The production of amino acids by electrical discharges in a "primitive" atmosphere.

In the illustration the gases used by Miller are shown. Our first experiment employed these same gases. As a result the production of glycine, alanine, and sarcosine described by Miller was verified. In later experiments the reaction chamber was reduced from 5 l to 1 l to cut the necessary irradiation period.

In the operation of the apparatus an electrical discharge and its associated ultraviolet light cause chemical changes in the reaction flask. Condensing steam in the form of water carries products out of the reaction vessel and into the smaller bulb below. The contents of the bulb are con-





FIG. 9. Amino acids formed in an atmosphere consisting of  $\text{CO}_2$ - $\text{N}_2$ - $\text{H}_2$ - $\text{H}_2\text{O}$ .

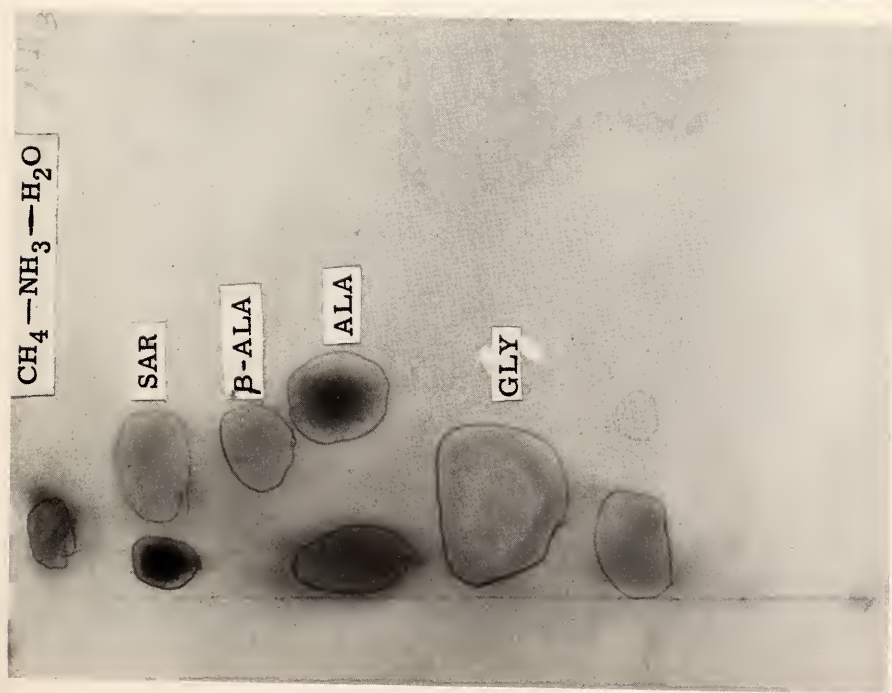


FIG. 8. Amino acids produced by electrical discharges in an atmosphere consisting of  $\text{CH}_4$ - $\text{NH}_3$ - $\text{H}_2\text{O}$ . Ala, alanine;  $\beta$ -ala,  $\beta$ -alanine; gly, glycine; sar, sarcosine.

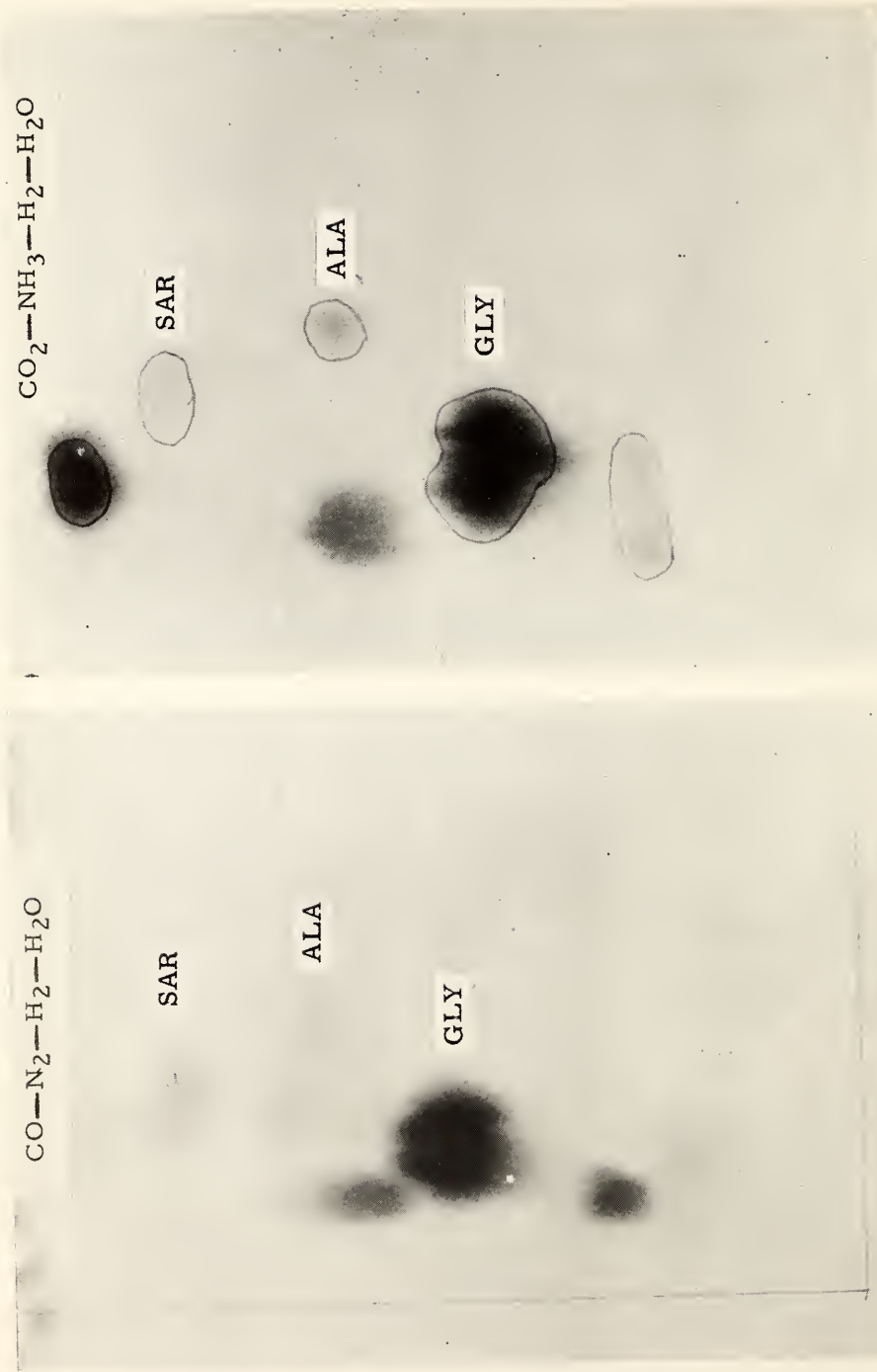


FIG. 10. Amino acids produced by electrical discharges in an atmosphere consisting of  $\text{CO}-\text{N}_2-\text{H}_2-\text{H}_2\text{O}$ . Ala, alanine; gly, glycine; sar, sarcosine.

FIG. 11. Amino acids formed in an atmosphere consisting of  $\text{CO}_2-\text{NH}_3-\text{H}_2-\text{H}_2\text{O}$ .

tinuously boiling. The less volatile products, such as amino acids, are retained, while the volatile substances are carried by steam back into the reaction chamber. With this apparatus, amino acids have been made in sizable amounts from widely differing initial gaseous compositions, including  $\text{CO}_2\text{—N}_2\text{—H}_2\text{O—H}_2$ ,  $\text{CO}_2\text{—NH}_3\text{—H}_2\text{O—H}_2$ ,  $\text{CH}_4\text{—N}_2\text{—H}_2\text{O}$ ,  $\text{CO—N}_2\text{—H}_2\text{O—H}_2$  as shown in table 9.

Studies involving  $\text{CO}_2$  as the source of carbon were facilitated through use of  $\text{C}^{14}\text{O}_2$ . It was possible to determine the amount of carbon appearing in various

acidic compounds and amphoteric, such as amino acids, were eluted with 0.1 *n* HCl. Most of the visible color of the original fluid was retained on the resin. The HCl eluate was placed on a Dowex-50 column in the  $\text{H}^+$  form, and the resin was washed with water. Acidic compounds were thus eluted. Finally, amino acids were removed from the column with  $\text{NH}_4\text{OH}$ .

Results of such a fractionation in some experiments employing  $\text{C}^{14}\text{O}_2$  are shown in table 10. In all runs except 5, the gaseous carbon compound has been abundantly fixed into a variety of compounds. The ex-

TABLE 9  
COMPOSITION OF "PRIMITIVE" ATMOSPHERES  
(Partial pressures of constituents in centimeters of mercury)

Run	$\text{CO}_2$	CO	$\text{CH}_4$	$\text{NH}_3$	$\text{N}_2$	$\text{H}_2$	$\text{H}_2\text{O}$	Duration of run (days)
1 .....	..	..	15	15	..	..	45	6
2 .....	..	..	20	..	10	..	40	11
3 .....	11	..	..	11	..	43	3	4
4 .....	9	..	..	18	..	25	3	6
5 .....	9	..	..	..	9	34	40	6
6 .....	9	..	..	..	9	34	3	6
7 .....	7	..	..	..	7	34	3	7
8 .....	..	11	..	..	6	42	3	18

compounds without using lengthy isolation and combustion procedures. In addition, tracer  $\text{C}^{14}\text{O}_2$  was employed in the  $\text{CO—N}_2\text{—H}_2$  experiment. In the presence of ultraviolet light,  $\text{CO}_2$  is readily dissociated to CO. Hence, the radioactive isotope can be expected to mirror the fate of the CO.

The aqueous solution in the boiling flask was sampled periodically, to monitor its composition. The major changes occurred in the first four days of operation.

At the conclusion of the run the product was fractionated by means of resin chromatography. The fluid was first placed on Dowex-2 in the OH form, and the resin was washed with water. Under these conditions bases are eluted, with some other compounds possessing no acid groups. The

ception is interesting, for it illustrates an important effect of the partial pressure of water vapor in this system.

Chromatograms displaying some of the amino acid products are shown in figures 8–11. It can be noted that glycine is a principal constituent in each, and that sarcosine is also abundant. Lesser quantities of alanine and  $\beta$ -alanine were also formed. The relative quantities of these substances depend in part, of course, on the original gas composition. It is perhaps not surprising that longer carbon chains are easier to obtain with  $\text{CH}_4$  than with  $\text{CO}_2$  as the initial gas. Nevertheless, experience to date indicates that additional, more complicated amino acids could be obtained from  $\text{CO}_2\text{—N}_2\text{—H}_2\text{O—H}_2$  mixtures.

The role of water vapor in the reaction

flask is of some importance. Under the influence of ultraviolet radiation of wavelengths shorter than 2000 Å, water is split into  $H+OH$ . As a result of combination,  $H_2+H_2O_2$  is formed. However,  $H_2$  is difficult to split, very short wavelengths being required. On the other hand,  $H_2O_2$  is redissociated with comparative ease into  $OH$ , a powerful oxidizing agent.

It is interesting that Miller obtained good yields of amino acids from  $CH_4+NH_3$  but only in the presence of large partial pressures of water. The amino acids are,

water temperatures were 50° C or higher, an event probably of limited duration, geologically speaking.

On the contrary, atmospheric temperatures less than 50° C are favorable to formation of amino acids from a mixture containing  $CO_2-N_2-H_2$ .

It is perhaps trivial to argue these points at length, for both the Miller experiment and the present work can be criticized with respect to the degree to which they simulate actual primitive conditions. More realistic experiments would employ ultra-

TABLE 10  
PRODUCTS FORMED BY IRRADIATION OF "PRIMITIVE" ATMOSPHERES

Run	RADIOACTIVITY IN CTS/SEC $\times 10^{-4}$				
	Original $C^{14}O_2$	Total fixed nonvolatile	Basics	Acidics	Amphoterics (amino acids)
4 .....	16	15.6	3.5	5.1	3.8
5 .....	16	0.8	0.2	0.4	0.1
6 .....	16	15.2	4.7	5.0	2.0
7 .....	16	15.5	4.6	5.3	2.1
8 .....	16	15.8	5.2	5.7	2.5

of course, considerably more oxidized than the initial  $CH_4$ . It would appear that, if the original atmosphere of the earth were  $CH_4+NH_3$ , amino acid formation in gaseous phase occurred in this environment in appreciable amounts only as long as surface

violet radiation with a spectral distribution similar to that striking the top of the atmosphere. Furthermore, it would be desirable to examine effects of ultraviolet light on aqueous systems in the presence of clay and other mineral catalysts.

## SULFIDE SYSTEMS

Detailed geological observations of the various sulfide deposits in combination with carefully conducted petrographic studies have produced some information about the mode of occurrence of the different types of ores. We are unable to observe the process of ore formation in nature, however, and are forced to deduce this process solely from the final products observed. Such deductions are not unambiguous, and only qualitative alternatives can be proposed. In the laboratory, on the other hand, processes and products can be directly correlated. Fine structures such as exsolution lamellae can be reproduced and

correlated with the natural sulfides. Experimental research thus enables the field investigator to interpret more intelligently the mineral associations observed in nature.

The ore minerals of greatest importance to the field geologist lie in the ternary systems  $Cu-Fe-S$ ,  $Fe-Ni-S$ , and  $Fe-Zn-S$ . The first of these was studied by Merwin and Lombard (1937) and Greig, Jensen, and Merwin (Year Book No. 54, 1954-1955). Subsolidus relations in the  $Fe-Zn-S$  system were successfully studied in part by Kullerud (1953), and the progress of an investigation of the  $Fe-Ni-S$  system is described below.



During the past year systematic studies of the Fe—Ni—S system have met with considerable success. Minerals whose compositions fall within this ternary system are pyrite and marcasite ( $\text{FeS}_2$  polymorphs), pyrrhotite ( $\text{Fe}_{1-x}\text{S}$ ), bravoite ( $(\text{Fe,Ni})_2$ ), vaesite ( $\text{NiS}_2$ ), millerite ( $\text{NiS}$ ), pentlandite ( $(\text{Fe,Ni})_9\text{S}_8$ ), violarite ( $(\text{Ni}_2\text{Fe})\text{S}_4$ ), polydymite ( $\text{Ni}_3\text{S}_4$ ), and

The laboratory study so far has led to a general understanding of the pyrrhotite-pentlandite relationship. Complete knowledge of this, however, requires detailed study of the entire FeS—NiS— $\text{Ni}_3\text{S}_2$  system.

Studies are progressing successfully also on other parts of the Fe—Ni—S system. The NiS—NiS<sub>2</sub>, FeS—FeS<sub>2</sub>, and FeS<sub>2</sub>—

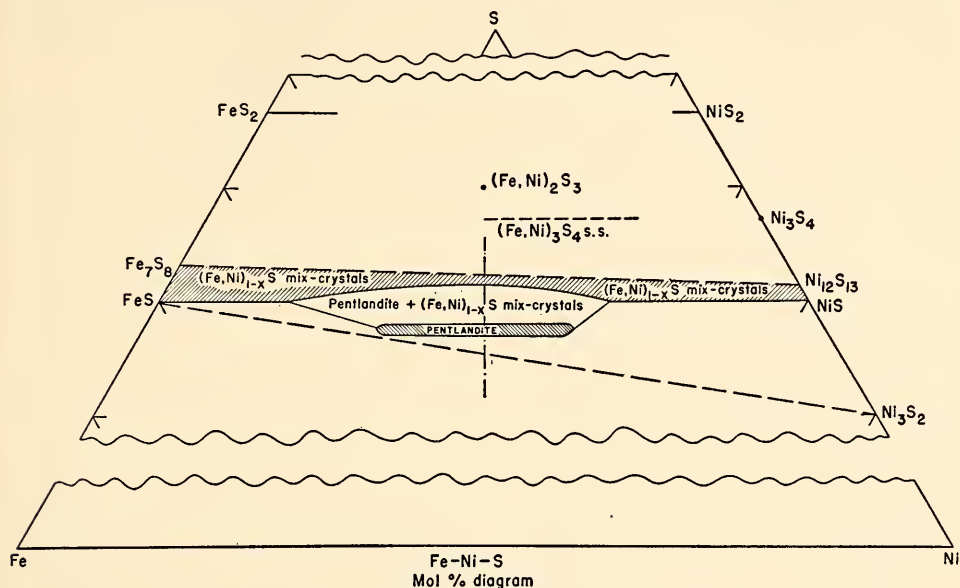


FIG. 12. The subsolidus phase relationships at 500° C for a part of the Fe—Ni—S system

heazlewoodite ( $\text{Ni}_3\text{S}_2$ ). Thus, this system includes some of the most common sulfide minerals (pyrite, marcasite, pyrrhotite) as well as pentlandite, the most important source of nickel.

In nature, pentlandite is nearly always intimately associated with pyrrhotite. This relationship, as exhibited in numerous ore deposits, has puzzled geologists for many years. The pentlandite-pyrrhotite assemblage occurs in basic rocks such as norites, and many geologists think that the sulfides were derived from the basic rocks by magmatic segregation. Well known examples of occurrences are the enormous deposits in the Bushveld district, South Africa, and in the Sudbury district in Canada.

NiS<sub>2</sub> joins are all being investigated systematically.

The minerals and mix-crystals in the ternary system are studied under sulfur pressures up to 5000 bars by a technique involving sealed gold tubes. The upper stability curve of pyrite has been determined by this method. The details of these various studies follow.

#### SUBSOLIDUS PHASE RELATIONS IN THE Fe—Ni—S SYSTEM

*Pentlandite—pyrrhotite* (Kullerud). Figure 12 shows the subsolidus phase relations at 500° C for a part of the Fe—Ni—S system. This preliminary diagram is based on about fifty runs across the section shown.

All runs were made in closed, evacuated silica tubes. At the middle left is FeS with the pyrrhotite omission solid solution series ending with  $\text{Fe}_7\text{S}_8$ . These pyrrhotites have the hexagonal NiAs-type structure, except below about  $140^\circ\text{C}$ , where atoms are arranged so as to form a supercell.

At the middle right on figure 12, NiS is seen with its omission solid solution series extending at  $500^\circ\text{C}$  to an approximate composition of  $\text{Ni}_{12}\text{S}_{13}$ . The low-temperature form of NiS, called millerite, inverts at  $374 \pm 4^\circ\text{C}$  to a high-temperature modification, which has the hexagonal NiAs structure and therefore is isostructural with compounds of the pyrrhotite series.  $\text{Fe}_7\text{S}_8$  and  $\text{Ni}_{12}\text{S}_{13}$  form a complete solid solution series above the inversion point. The solid solubilities of the stoichiometric compounds NiS in FeS, or vice versa, are very much temperature-dependent, but never complete. Thus, for instance, the solubility of NiS in FeS at  $500^\circ\text{C}$  is about 20 mol per cent, and the solubility of FeS in NiS is about 30 mol per cent. The solubilities of both sides increase markedly with increasing temperature.

When heated at  $500^\circ\text{C}$  for a minimum of 75 days, mixtures of FeS and NiS, in the region where NiS amounts to more than 20 mol per cent and less than 70 mol per cent, will produce pyrrhotite-type  $(\text{Fe},\text{Ni})_{1-x}\text{S}$  mix-crystals as well as pentlandite. This two-phase field is outlined in figure 12. From a number of runs it appears that the pentlandite cubic structure will tolerate a considerable variation in the Fe/Ni ratio with deviations on both sides of the commonly found 1/1 ratio. The exact boundaries of the pentlandite field have not yet been determined.

A vertical cut was made along the dash-and-dot line in figure 12. All phases that occur when the metal-to-sulfur ratio is varied from 7.5/8 to 9/8 were studied. In this cut the iron-to-nickel ratio is constant and equal to 1.

The results of these preliminary studies are brought together in figure 13. In the field above the liquidus curves *AE* and

*EB*, one melt exists. The field *ADE* contains pyrrhotite-type  $(\text{Fe},\text{Ni})_{1-x}\text{S}$  mix-crystals and liquid of composition as given by curve *AE*. In the field *BCE* are found pentlandite mix-crystals and liquid. Pyrrhotite-type  $(\text{Fe},\text{Ni})_{1-x}\text{S}$  mix-crystals alone occur in the field on the left, limited upward by the *AD* solidus curve and on the right by the sloping solvus curve.

The big field in the middle contains both pentlandite and  $(\text{Fe},\text{Ni})_{1-x}\text{S}$  mix-crystals, and pentlandite is found in the field to the right under the *BC* solidus curve.

In this section the end member on the left has a metal-to-sulfur ratio of about 7.5/8, and its melting point is approximately  $950^\circ\text{C}$ . The end member on the right side melts at about  $880^\circ\text{C}$ . The "eutectic" point *E* lies at a metal-to-sulfur ratio of about 7.92/8 and at a temperature of  $845 \pm 5^\circ\text{C}$ . The solvus curve starts at point *D* ( $845^\circ\text{C}$ ) at a metal-to-sulfur ratio of about 7.85/8. This ratio decreases with decreasing temperature, and is 7.6/8 at  $500^\circ\text{C}$ . The  $(\text{Fe},\text{Ni})_{1-x}\text{S}$  pyrrhotite-type mix-crystals thus require an increasing sulfur content with decreasing temperature.

A number of runs indicate a clear change in the metal-to-sulfur ratio of pentlandite with variation in temperature. This change, not yet completely determined, is indicated in figure 13. Starting with a melt having a metal-to-sulfur ratio of, for instance, 1/1, the *EB* curve will be intersected as the temperature is lowered, and pentlandite will start to crystallize from the melt. If these crystals are removed, as numerous geologists think happens in many ore deposits of this kind, the remaining melt will change in composition toward the "eutectic" *E*. Here all melt solidifies at once, and  $(\text{Fe},\text{Ni})_{1-x}\text{S}$  mix-crystals of composition *D* and pentlandite of composition *C* form. As we now decrease the temperature in the subsolidus region, the  $(\text{Fe},\text{Ni})_{1-x}\text{S}$  mix-crystals will require an increasing amount of sulfur. This demand is met by exsolution of pentlandite from the  $(\text{Fe},\text{Ni})_{1-x}\text{S}$  mix-crystals. Exsolution of  $(\text{Fe},\text{Ni})_{1-x}\text{S}$  mix-crystals from pentland-

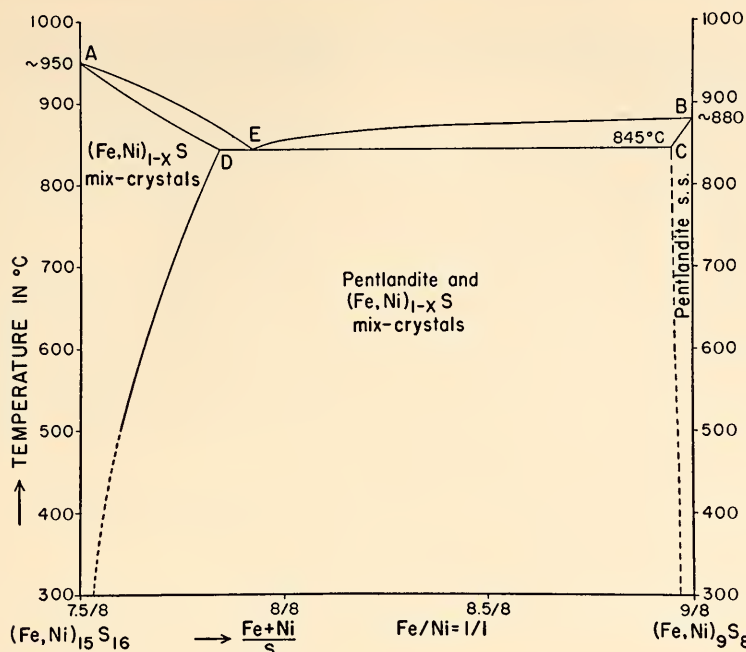


FIG. 13. A vertical cut in the Fe-Ni-S system. Here the nickel-to-iron ratio is constant and equal to 1, and the metal-to-sulfur ratio varies from 7.5/8 to 9/8.

ite has been observed to occur both in nature and in laboratory experiments. Therefore, pentlandite does change its metal-to-sulfur ratio to a certain extent in the solid state. This change has been indicated in figure 13. Investigations of this solvus curve are in progress.

*The pyrrhotite-pyrite relationship* (Arnold). The pyrrhotite ( $\text{Fe}_{1-x}\text{S}$ )-pyrite ( $\text{FeS}_2$ ) mineral assemblage is of common occurrence in many sulfide ore deposits. The subsolidus relations between coexisting pyrite and pyrrhotite are now being studied at temperatures ranging from 300° to 700° C. All runs are made in evacuated, closed silica tubes under sulfur vapor pressures ranging from a few millimeters of mercury to about 1 atm. The composition of pyrrhotite in equilibrium with pyrite has been found to vary appreciably with temperature.

Pyrrhotite, well known for its ability to omit iron from its hexagonal lattice, will when heated at 800° C with free sulfur be of composition  $\text{Fe}_{0.67}\text{S}$ . The time needed for

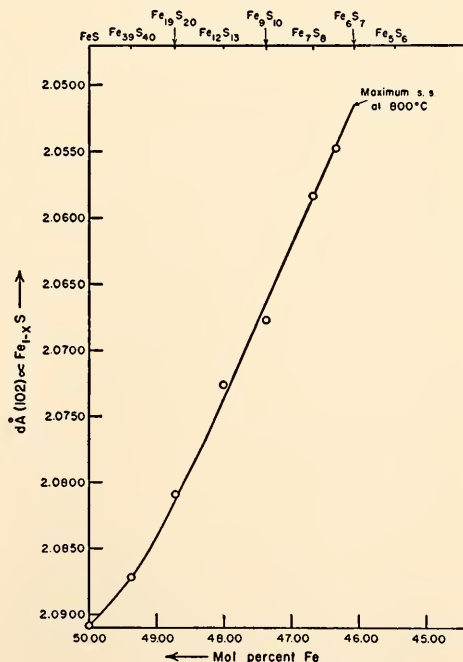


FIG. 14. Curve showing the relationship between  $d$  spacings for the (102) X-ray reflections and composition of synthetic pyrrhotites.

equilibrium to be established between the pyrite and pyrrhotite phases is, even at 600° C, more than 75 days.

X-ray diffraction studies of pyrrhotites ranging in composition from  $\text{FeS}$  to  $\text{Fe}_{6.5}\text{S}_7$  have shown an appreciable variation in the (102)  $d$  spacing between the mentioned compositions. The (102)  $d$  spacings versus composition have been plotted in figure 14. Silicon was used as an internal standard for all measurements. By this method pyrrhotite compositions may be determined as accurately as by chemical methods, and more conveniently.

Pyrite formed in equilibrium with pyrrhotite at temperatures from 300° to 700° C showed no detectable change in its unit

cell dimensions ( $a=5.419\pm0.002$  Å). The results so far obtained demonstrate that the composition of the pyrrhotite mix-crystals formed in equilibrium with pyrite varies sufficiently with changes in temperature to provide a sensitive temperature scale suitable for application to natural pyrrhotite-pyrite assemblages.

*The NiS—NiS<sub>2</sub> join* (Arnold, Kullerud). NiS was prepared dry from nickel and sulfur. Vaesite ( $\text{NiS}_2$ ) forms readily from NiS and sulfur heated together at 500° C. Nickel sulfide of the hexagonal NiAs structure type forms a solid solution series in which the metal-to-sulfur ratio varies in

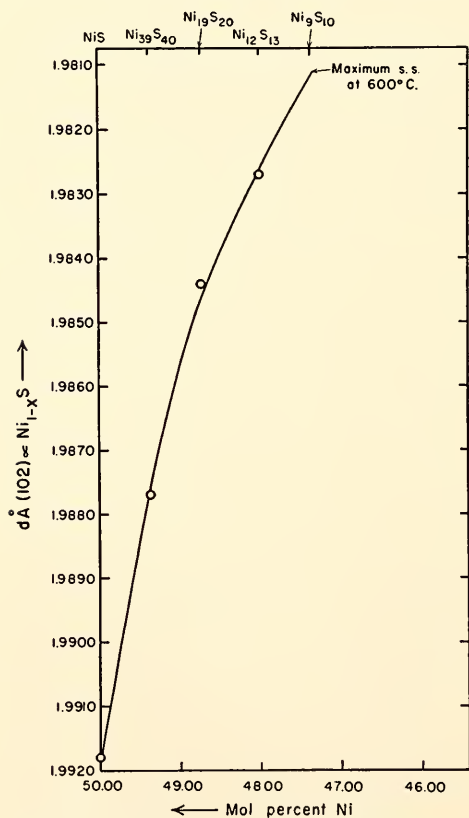


FIG. 15. Curve showing variation in  $d$  spacings for the (102) X-ray reflections as function of mix-crystal ( $\text{Ni}_{1-x}\text{S}$ ) composition.

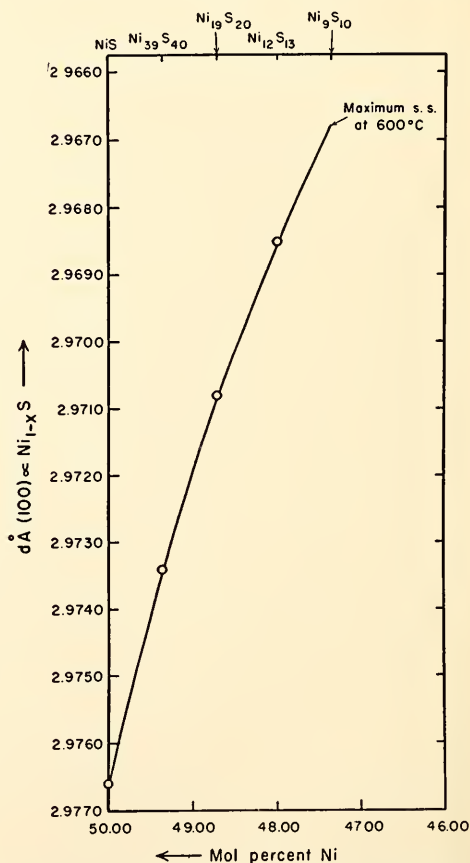


FIG. 16. Curve showing variation in  $d$  spacings for the (100) X-ray reflections as function of mix-crystal ( $\text{Ni}_{1-x}\text{S}$ ) composition.



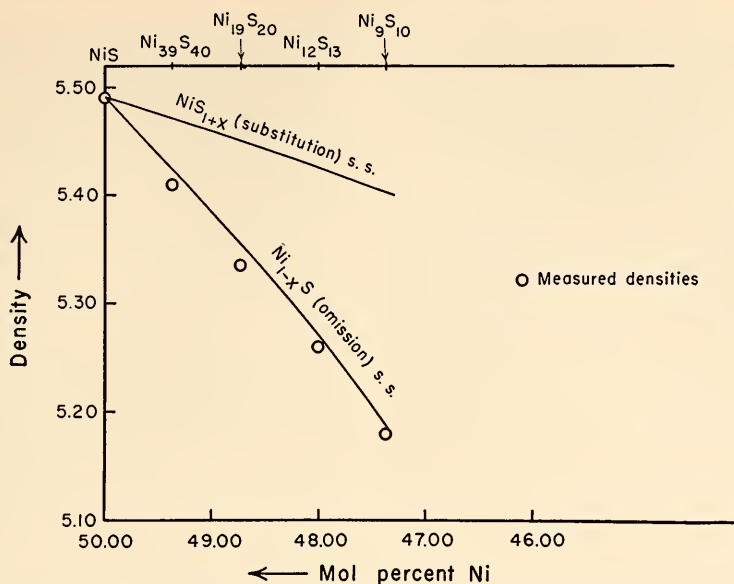


FIG. 17. Upper curve shows calculated densities for substitutional solid solution. Lower curve shows calculated densities for omission-type solid solution. Experimentally determined densities agree with calculated omission solid solution densities.

much the same way as that for the pyrrhites. In order to study this solid solution series,  $\text{NiS}$ ,  $\text{Ni}_{39}\text{S}_{40}$ ,  $\text{Ni}_{19}\text{S}_{20}$ , and  $\text{Ni}_{12}\text{S}_{13}$  were synthesized at  $600^\circ\text{C}$ . The variation in the  $2\theta$  values of the (100) and (102) reflections was studied by X-ray diffraction spectrometer methods. The corresponding variations in the  $d$  spacings for the (102) and (100) reflections versus composition are shown in figures 15 and 16. The end member of the solid solution series at  $600^\circ\text{C}$  is seen from the figures to be a compound of composition  $\text{Ni}_9\text{S}_{10}$ .

Unit cell dimension data obtained as mentioned above were used for calculations of specific gravities for the different compounds. The upper curve in figure 17 shows calculated densities for substitution-type solid solution, and the lower curve shows calculated densities for omission-type solid solution. The experimentally determined densities (Berman balance and pycnometer) are in very good agreement with the densities calculated for omission

solid solution. This solid solution series is of the omission type and should be expressed by the formula  $\text{Ni}_{1-x}\text{S}$ .

$\text{FeS}_2$ — $\text{NiS}_2$  (Kullerud). Pyrites containing appreciable amounts of nickel are fairly common in nature. These pyrite-type  $(\text{Fe},\text{Ni})\text{S}_2$  mix-crystals have been regarded as separate minerals, with separate mineral names. It is the object of the present study to determine the solid solubility of nickel in pyrite as well as that of iron in vaesite at varying temperatures.

Vaesite and pyrite are isostructural. Under 1 atm of sulfur vapor pressure, pyrite is stable up to about  $690^\circ\text{C}$  and vaesite up to  $780^\circ\text{C}$ . It has so far been indicated by a number of runs, which lasted 80 days, that the solubility of Ni in  $\text{FeS}_2$ , as well as that of Fe in  $\text{NiS}_2$ , is appreciably less than 10 per cent at  $550^\circ\text{C}$ .

$\text{NiS}$  (Eugster, Kullerud). Of the two  $\text{NiS}$  polymorphs only the low ( $\beta$ ) form, called millerite, has been found in nature. The high-temperature modification has the hexagonal  $\text{NiAs}$  atomic structure. It is

reported in the literature that the  $\alpha$ - $\beta$  inversion takes place at  $396^\circ\text{C}$ . This temperature was obtained from heating experiments which are not well suited for determination of inversion points because of time lag in the inversion.

The temperature of inversion was established by a series of quenching runs performed in evacuated, closed silica tubes. The  $\alpha$ - $\beta$  inversion of pure NiS takes place at  $374^\circ \pm 3^\circ\text{C}$ . A number of runs were also undertaken in order to investigate the influence of pressure on this  $\alpha$ - $\beta$  inversion. The  $P$ - $T$  curve is very steep. The inversion takes place below  $400^\circ\text{C}$  even at 2000 bars.

The literature shows a good deal of disagreement on the melting point of NiS, values anywhere between  $790^\circ$  and  $990^\circ\text{C}$  having been given. As carefully determined by heating-curve experiments it was found to be  $800.5^\circ \pm 1.0^\circ\text{C}$ .

#### THE $\text{Cu}_2\text{S}$ — $\text{CuS}$ SYSTEM

G. KULLERUD

Further studies have shown that covellite ( $\text{CuS}$ ) is stable up to  $235^\circ\text{C}$ , at which temperature it breaks down to digenite ( $\text{Cu}_9\text{S}_5$ ) and free sulfur. Digenite in turn is stable up to about  $550^\circ\text{C}$ , at which temperature it breaks down to chalcocite ( $\text{Cu}_2\text{S}$ ) and free sulfur. The breakdown of covellite at  $235^\circ\text{C}$  is in disagreement with results obtained by various earlier workers. Heating-curve experiments on covellite kept in an evacuated, closed silica tube with a deep thermocouple well indicate that the breakdown occurs at about  $490^\circ\text{C}$ . Long runs with copper and sulfur in the ratio 1/1 at  $245^\circ$ ,  $260^\circ$ ,  $300^\circ$ , and  $350^\circ\text{C}$ , however, all produce digenite and free sulfur. The same results are obtained when mixtures of copper and sulfur in the ratios 8/5, 7/5, and 6/5 are heated together for 30 days at  $250^\circ$ ,  $300^\circ$ , and  $350^\circ\text{C}$ . When the mixtures of copper and sulfur in the same ratios as mentioned above are heated

at  $225^\circ$ ,  $200^\circ$ ,  $150^\circ$ , and  $90^\circ\text{C}$  for 100 days, digenite and covellite are produced together. Variations in the unit cell dimensions of these covellites produced at  $90^\circ$ ,  $150^\circ$ ,  $200^\circ$ , and  $225^\circ\text{C}$  in equilibrium with digenite indicate a measurable extent of solid solution of copper in  $\text{CuS}$ . The digenite produced in equilibrium with covellite in these runs shows very little change in the unit cell dimensions with changing temperatures. A number of runs have indicated, however, that a digenite solid solution may extend to  $\text{Cu}_{8.5}\text{S}_5$  at  $500^\circ\text{C}$ . The digenite solid solution series does not extend measurably beyond the  $\text{Cu}_9\text{S}_5$  composition on the chalcocite side. When copper and sulfur in the ratio 9.2/5 are heated together for 30 days at  $300^\circ\text{C}$ , digenite and chalcocite are produced. On the other hand, in some runs chalcocite-type solid solution has been indicated to extend to  $\text{Cu}_{9.5}\text{S}_5$  above  $350^\circ\text{C}$ . High chalcocite inverts to low chalcocite at  $90^\circ \pm 5^\circ\text{C}$ . This inversion is very rapid, and it is, therefore, not possible to quench high chalcocite. The study of high chalcocite solid solutions, accordingly, becomes somewhat difficult.

#### $\text{CuFeS}_2$ — $\text{ZnS}$

G. KULLERUD

The coexistence of chalcopyrite ( $\text{CuFeS}_2$ ) and sphalerite ( $\text{ZnS}$ ) is very common in nature. Chalcopyrite occurs in two polymorphs. The low form is tetragonal, and the high form is cubic of the sphalerite type. Extensive solubility has been found to exist between high chalcopyrite and sphalerite. Thus at  $600^\circ\text{C}$  almost 40 per cent  $\text{CuFeS}_2$  dissolves in  $\text{ZnS}$ .

In agreement with earlier findings by Merwin and Lombard (1937), the present study has shown that chalcopyrite is a sulfur-deficient compound. It has not been possible to make the stoichiometric  $\text{CuFeS}_2$  compound under laboratory conditions. The amount of sulfur deficiency of the compounds depends on their temperature

of formation. Thus at  $700^{\circ}\text{C}$  a compound of composition  $\text{CuFeS}_{1.92}$  is stable.

The mentioned inversion in chalcopyrite has been explained by other workers to be one of order-disorder of the metal atoms. The temperature at which inversion takes place has now been found to depend on the composition of the chalcopyrite.

### THE UPPER STABILITY CURVE OF PYRITE

G. KULLERUD AND H. S. YODER, JR.

Pyrite is the most common sulfide occurring with rock-forming silicates. Knowledge of its stability field, therefore, would contribute to an understanding of the conditions existing during formation of common rocks.

Pyrite may be readily synthesized in the dry way in silica tubes by mixing troilite or pyrrhotite with appropriate amounts of sulfur. In this manner pyrite was crystallized at temperatures from  $350^{\circ}\text{C}$  to  $700^{\circ}\text{C}$ . The sulfur vapor pressure in the silica tubes varied from a few millimeters of mercury at  $350^{\circ}\text{C}$  to about 40 bars at  $700^{\circ}\text{C}$ . It was desirable to obtain data at the higher pressures believed to exist in the earth's crust, and a different experimental technique was required.

Considerable success has been attained in the synthesis of hydrous minerals at high water pressures by various methods. The familiar technique of using sealed collapsible tubes appeared to be readily adaptable to sulfides if a ductile material which would withstand the corrosive action of sulfur could be found. Platinum tubing, normally used in hydrothermal work, was not desirable for these experiments because iron and platinum, combined, readily form sulfides stable to at least  $900^{\circ}\text{C}$ . Sulfides of gold, however, break down below  $300^{\circ}\text{C}$ . Sealed gold tubes, therefore, were tried and found well suited for these experiments. In no case was the gold tubing noticeably attacked by the sulfur vapor in the range of conditions of this study.

The runs up to 2000 bars were carried out in "cold seal" bombs, and those at 5000

bars in Yoder's internally heated bomb. At least two gold tubes were run simultaneously in order to approach equilibrium from two different mixtures. One tube contained synthetic troilite or pyrrhotite plus sulfur, and the second tube contained synthetic pyrite.

The confining pressure is applied to the outside of the tubes by means of water or argon. The sulfur vapor pressure in these tubes in each run was believed to be essentially equal to the confining pressure

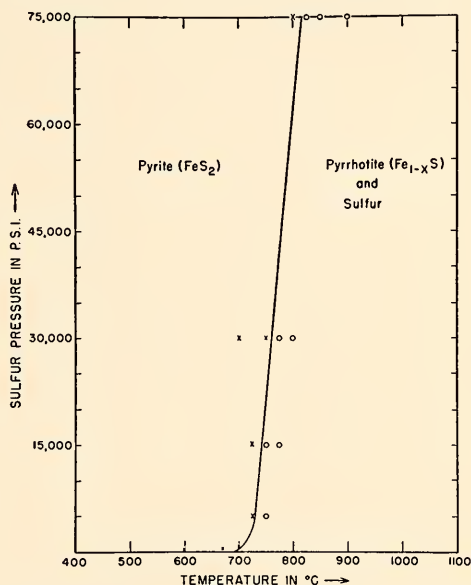


FIG. 18. The upper stability curve of pyrite

because of the collapse of the tubes. All runs took place well above the critical pressure of sulfur (about 116 bars).

The upper stability curve of pyrite is shown in figure 18. Points on the curve are:  $690^{\circ}\text{C}$ , at 1 atmosphere sulfur vapor pressure, as given by Allen and Lombard in 1917;  $730^{\circ}\text{C}$ , at 5000 psi;  $745^{\circ}\text{C}$ , at 15,000 psi;  $760^{\circ}\text{C}$ , at 30,000 psi; and  $815^{\circ}\text{C}$ , at 75,000 psi.

Pyrite breaks down to a pyrrhotite solid solution and sulfur. In the diagram the stability field of pyrite is on the left of the curve and the field of pyrrhotite and sulfur is on the right.

The lower stability limit of pyrite has not yet been determined; experiments indicate, however, that marcasite, if it really is an  $\text{FeS}_2$  polymorph, is stable at temperatures well below  $350^\circ\text{C}$ .

Pyrite grows as pale brass-yellow crystals; a preliminary study of the morphology of these cubic crystals has shown that cubes, octahedra, pyritohedra, and dodecahedra occur (see fig. 19). No definite relation has been established between crystal habit and  $P$ - $T$  conditions of crystal growth.

Powder X-ray diffraction studies of pyrite grown under varied pressure and temperature conditions (from  $350^\circ\text{C}$  at a few millimeters of mercury to  $800^\circ\text{C}$  at 5000 bars) show no measurable variation in the cell length of the pyrite crystals. The length  $a$  is always within the limits  $5.420 \pm 0.002 \text{ \AA}$ .

The composition of the pyrrhotite breakdown product varies somewhat with temperature and pressure. Increase in temperature under constant pressure causes a decrease in the metal-to-sulfur ratio. An increase in pressure at a constant temperature, on the other hand, appears to cause an increase in the metal-to-sulfur ratio. Powder X-ray diffraction studies of pyrrhotites formed under various pressure and temperature conditions from  $750^\circ\text{C}$  at 335 bars to  $900^\circ\text{C}$  at 5000 bars show that the composition varies between 53.2 and 53.9 mol per cent sulfur (or roughly between  $\text{Fe}_8\text{S}_9$  and  $\text{Fe}_6\text{S}_7$ ).

Since pyrite breaks down to a pyrrhotite mix-crystal (the composition of which varies with pressure and temperature) plus sulfur, it might be asked whether the upper stability of pyrite can be sharply defined by a uniform curve. The decomposition of pyrite at any pressure occurs at a definite temperature, and the composition of the pyrrhotite is thereby determined. The composition of the pyrrhotite solid solution at the breakdown of pyrite is given by the intersection of the pyrrhotite solvus curve with the solidus in the system  $\text{FeS}$ - $\text{S}$ , and thus contains the maximum amount of sulfur.

Pyrrhotite in these runs occurs as almost

black magnetic crystals having a hexagonal prismatic habit. The sulfur formed by decomposition of pyrite appears white to light yellow. Sulfur from runs at various pressures and temperatures was X-rayed at room temperature and found to be of the orthorhombic form.

In figure 20 the upper stability curve of pyrite is shown in relation to the beginning-of-melting curve of a natural tho-

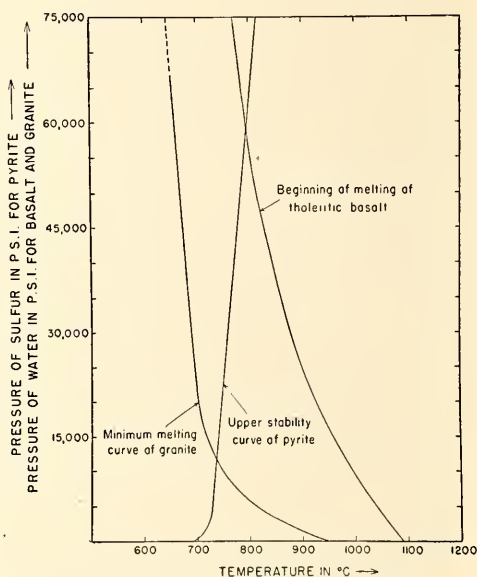


FIG. 20. The upper stability curve of pyrite shown in relation to the beginning-of-melting curve of natural tholeiitic basalt and the minimum-melting curve of granite.

leitic basalt and the minimum-melting curve of granite. The basalt curve has recently been determined by Yoder and Tilley; the melting curve of granite is from the well known work of Bowen and Tuttle.

The occurrence of primary pyrite in amphibolites and granites and not in basalts and rhyolites is accounted for by comparison of these curves. It must be pointed out that the upper stability curve of pyrite was worked out where the sulfur pressure was equal to the total pressure, and the curves for beginning of melting of tholeiitic basalt and for minimum melting of granite were determined where the water pressure



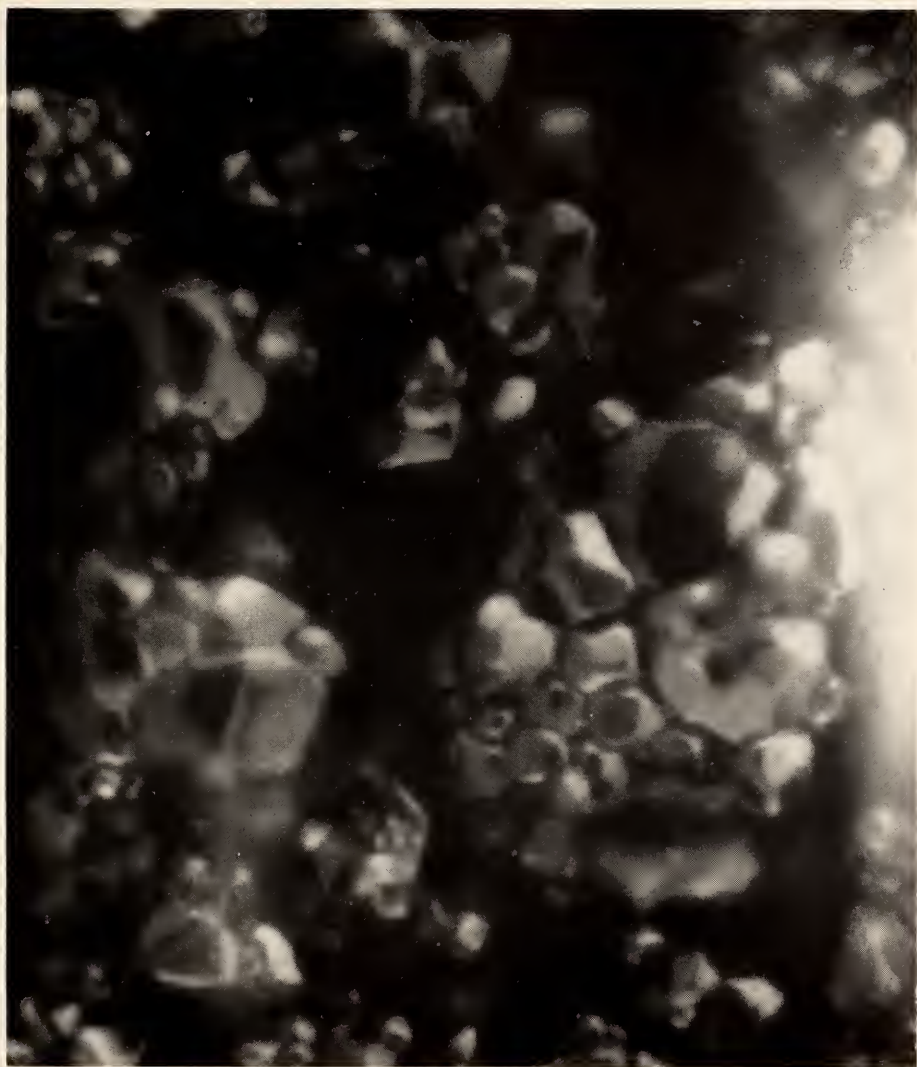


FIG. 19. Pyrite crystals grown at  $725^{\circ}$  C under 2000 bars of sulfur pressure. Cubes, octahedra, pyritohedra, and dodecahedra are formed at this pressure and temperature.  $\times 400$ .



was equal to the total pressure. These general relations also hold for sulfur pressures much less than the total pressure,

however, as may be seen from the small increase in the stability of pyrite with increasing pressure.

## ROUTINE ABSOLUTE MEASUREMENT OF BETA RADIOACTIVITY AT LOW LEVEL FROM THICK SAMPLES

W. F. LIBBY

Suttle and Libby (1955), in pursuit of earlier leads (Libby, 1947), showed that beta radioactivities which involve transitions between only two states rather than a mixture of such transitions display exponential absorption when the sample is cylindrically placed closely around the detecting cylindrical Geiger counter even though the transition may be highly forbidden as in the case of naturally radioactive potassium,  $K^{40}$ . Since under conditions of exponential absorption (and consequently self-absorption) the effective thickness of a sample, thick with respect to the penetrating power of the radiation, is precisely the reciprocal of the absorption coefficient, absolute assay requires only a

correction factor for geometry,  $G$ , and for absorption in the counter wall. Suttle and Libby used this method to determine the half-life of  $K^{40}$ , obtaining close agreement with the value they obtained by more conventional but much more laborious methods of absolute assay.

Additional data have been obtained for the isotopes marked with asterisks in table 11; a general relation between the absorption coefficient and the maximum energy of the beta radioactivity shown in figure 21 has been discovered; and further confirmation has been obtained for the Lerch (1953) relation for the dependence of absorption coefficients on atomic weight of the absorber as shown in figure 22. With these

TABLE 11  
BETA-RAY ABSORPTION DATA

Isotope	Maximum energy of beta spectrum (Mev)	Absorbing material	Half-thickness (mg/cm <sup>2</sup> )	Reciprocal of absorption coefficient (mg/cm <sup>2</sup> )
T	0.0189	He	0.050	0.0720
Zr <sup>98</sup>	0.060	Al	0.35	0.506
Sn <sup>151</sup>	0.0755	Al	0.63	0.91
C <sup>14</sup>	0.155	Al	1.9	2.74
S <sup>35</sup> *	0.167 *	Mylar plastic *	2.2 *	3.16 *
		Al *	2.3 *	3.3 *
		Mylar *	2.7 *	3.9 *
Rb <sup>87</sup>	0.270	Al	4.85	7.0
Ca <sup>45</sup> *	0.255 *	Al *	4.9 *	7.1 *
Tc <sup>99</sup>	0.296	Al	6.09	8.8
Tl <sup>204</sup>	0.762	Al	22	32
Cl <sup>36</sup> *	0.716 *	Al *	32 *	46 *
		Cu *	26 *	37 *
		Sn *	21 *	30 *
		Sn *	18 *	26 *
K <sup>40</sup>	1.36	Al	67.0	96
P <sup>32</sup>	1.708	Al	84	122
		Cu *	60 *	86 *
		Sn *	50 *	72 *
Y <sup>90</sup> *	2.275 *	Al *	130 *	189 *

\* New data.

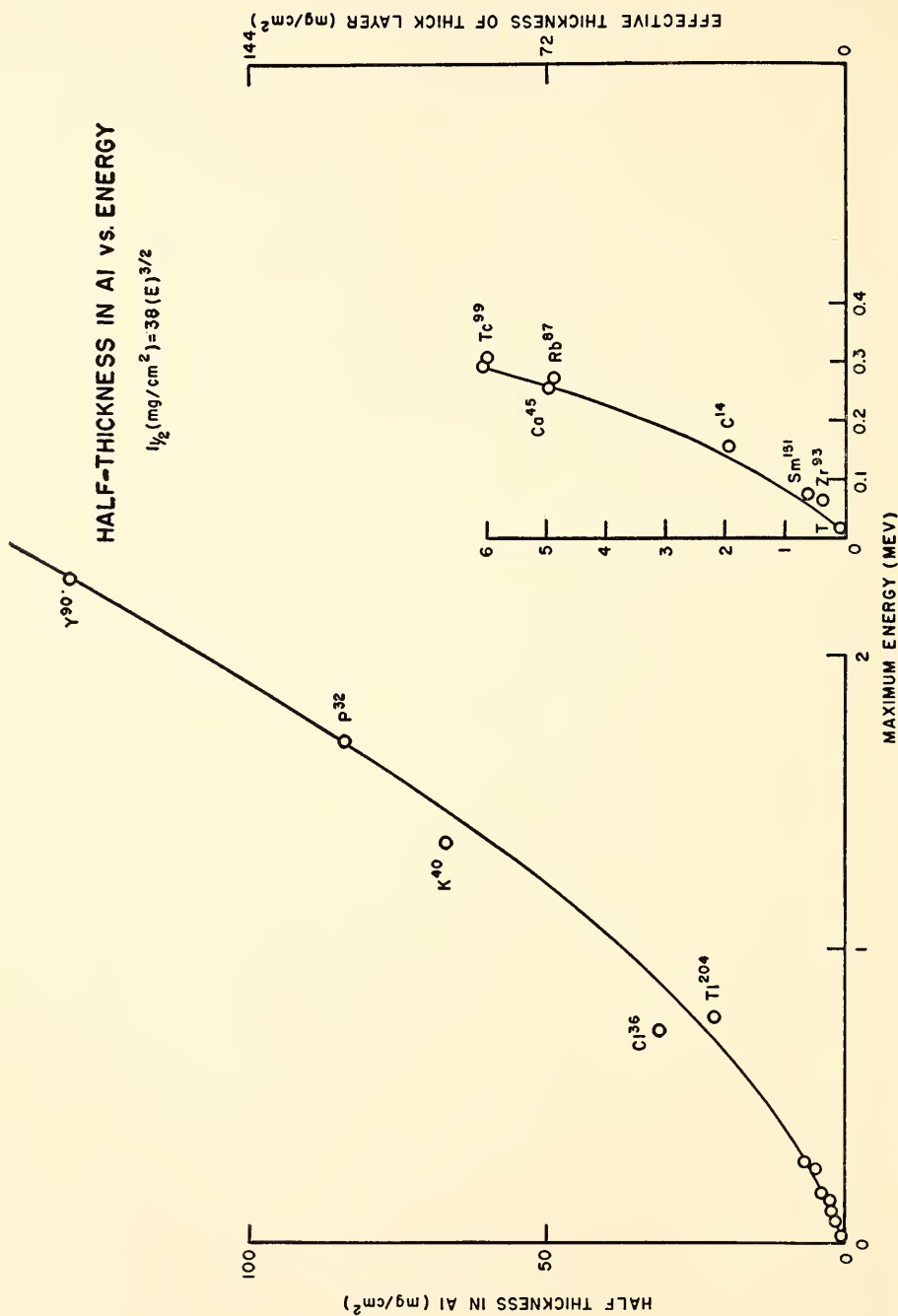


FIG. 21. Absorption half-thickness in aluminum versus beta energy



# HALF-THICKNESS vs. ATOMIC WEIGHT OF ABSORBER

SOLID CURVE: 
$$\frac{1/2}{(1 + \frac{A_t \cdot W_t}{100})} = \frac{(1/2)_\text{Al}}{(1 + \frac{27}{100})}$$

- C<sup>14</sup> 155 kev
- ⊠ S<sup>35</sup> 167 kev
- Cl<sup>36</sup> 716 kev
- ⊙ Ti<sup>204</sup> 770 kev
- x P<sup>32</sup> 1,708 mev

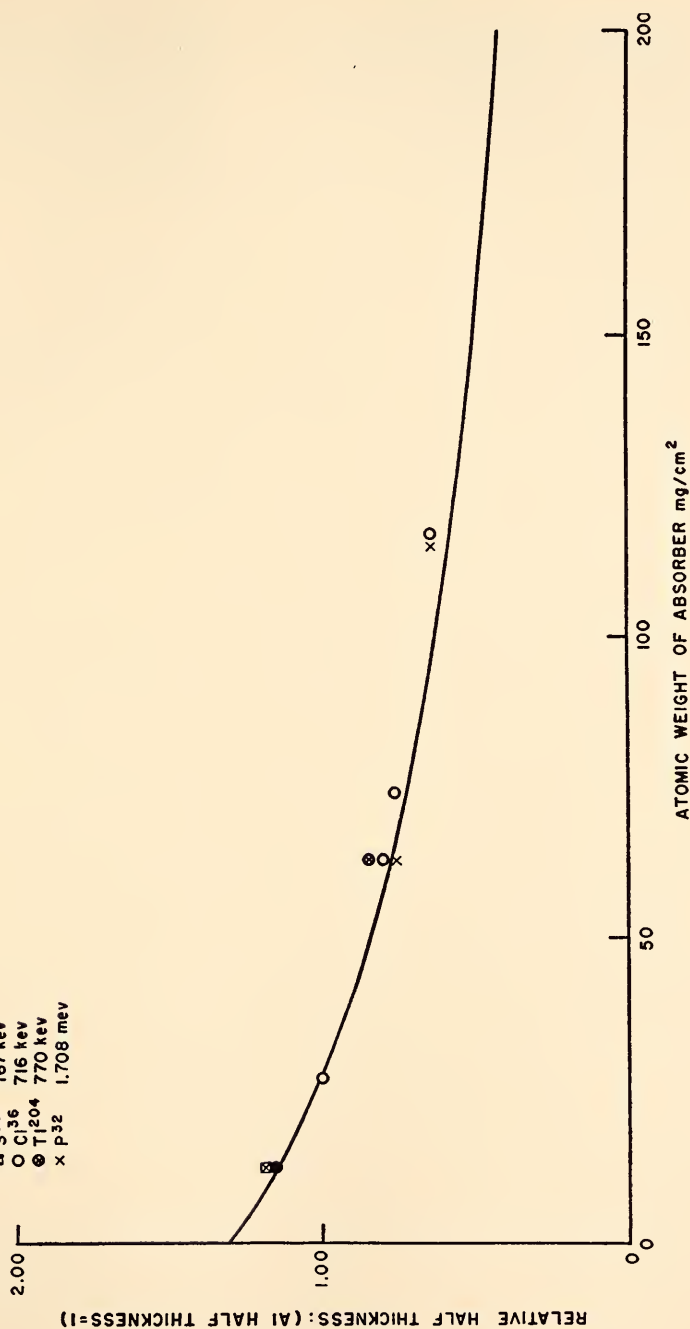


Fig. 22. Half-thickness versus atomic weight of absorber

results a general expression can be written for the absolute specific activity,  $\sigma$ , of a solid (or liquid) thick with respect to  $\lambda$  (reciprocal of absorption coefficient) in terms of the count rate  $R$  (counts per minute) observed from an area  $A$  of it in close cylindrical contact with a counter of wall thickness  $l$  and wall material of atomic weight  $M$  if the average solid angle subtended by the sensitive volume of the counter at the sample is on the average  $4\pi/\bar{G}$  and the atomic weight and atomic number of the sample are known to be  $M$  and  $Z$ , and the upper energy limit of the single beta transition spectrum involved is  $E$  (Mev), as

$$\sigma \frac{\text{dpm}}{\text{mg}} = \frac{R(\text{cpm})}{A(\text{cm}^2)} \frac{\bar{G} \exp \left\{ \frac{l}{70E^{3/2}} \left( 1 + \frac{M}{100} \right) \right\}}{55E^{3/2} \left( 1 + 0.65 \left[ 1 - \exp \left\{ -\frac{Z}{35} \right\} \right] \right)},$$

where the term  $0.65 [1 - \exp \{-Z/35\}]$  represents the back-scattering coefficient given by Glendenin and Solomon (1950), the factor  $1/2$  arising because the back-scattered radiation has approximately half the

penetrating power of the original.

This methodology probably is accurate to about 5 per cent and is particularly applicable to low specific activities, since the material should be placed close to the cylindrical counter to ensure exponential absorption and this arrangement gives the maximum sensitivity. Thus simple absolute assay of low-level samples is possible to about 5 per cent accuracy for all beta-emitting isotopes involving single transitions. These include nearly all isotopes of practical interest to industry and medicine, where the premium for keeping quantities of radioactivity so small as to be entirely

safe is enormous. With these procedures and methods of low-level counting, many important everyday applications of isotopes in industry and medicine should be possible.

## FELDSPARS AND FELDSPATHOIDS

### STUDIES IN THE SYSTEM $\text{NaAlSi}_3\text{O}_8\text{—H}_2\text{O}$

W. S. MacKENZIE

One of the important aspects of experimental petrology is the synthesis of minerals with properties similar to those of natural minerals, and the determination of their stability. Recent phase-equilibrium studies carried out at extreme pressures have shown that it is possible to synthesize many of the minerals that had hitherto not been synthesized in the laboratory. No one, however, has succeeded in crystallizing either low-temperature albite or microcline, and yet there is no reason to believe that any special conditions of extreme pressure or shearing stress are necessary for the formation of either in nature.

Crystallization of a glass of composition  $\text{NaAlSi}_3\text{O}_8$  in the presence of water or some other suitable flux produces crystals that differ markedly in physical properties from the common naturally occurring al-

bite of pegmatites, granites, and some metamorphic rocks. The studies in the system  $\text{NaAlSi}_3\text{O}_8\text{—H}_2\text{O}$  reported here are concerned only with the nature of the crystalline material, and are aimed at finding a method of crystallizing the low-temperature form of albite. At the same time it is hoped that more light will be shed on the nature of the transformation of the low-temperature form to the high-temperature form to supplement the studies being made by J. R. Smith on the effects of heating plagioclases.

Because the albite crystals synthesized were too small for determination of optical properties, powder X-ray diffraction methods were used exclusively to determine the nature of the crystals. Tuttle and Bowen showed that high-temperature albite could be distinguished readily from low-temperature albite by the separation of the  $131$  and  $\bar{1}\bar{3}1$  reflections in X-ray powder patterns, and the value  $2\Theta_{131} - 2\Theta_{\bar{1}\bar{3}1}$  has been

used throughout the present study to characterize the crystalline products of each experiment, since it was found that this angular difference is very sensitive to the conditions of crystallization. More than 300 runs have been made crystallizing a

glass of composition  $\text{NaAlSi}_3\text{O}_8$  in the presence of water vapor in Tuttle cold-seal pressure vessels. Figure 23 shows a plot of the value of  $2\Theta_{131} - 2\Theta_{1\bar{3}1}$  against time of duration of the experiment for a few of the runs made. The water vapor pressure

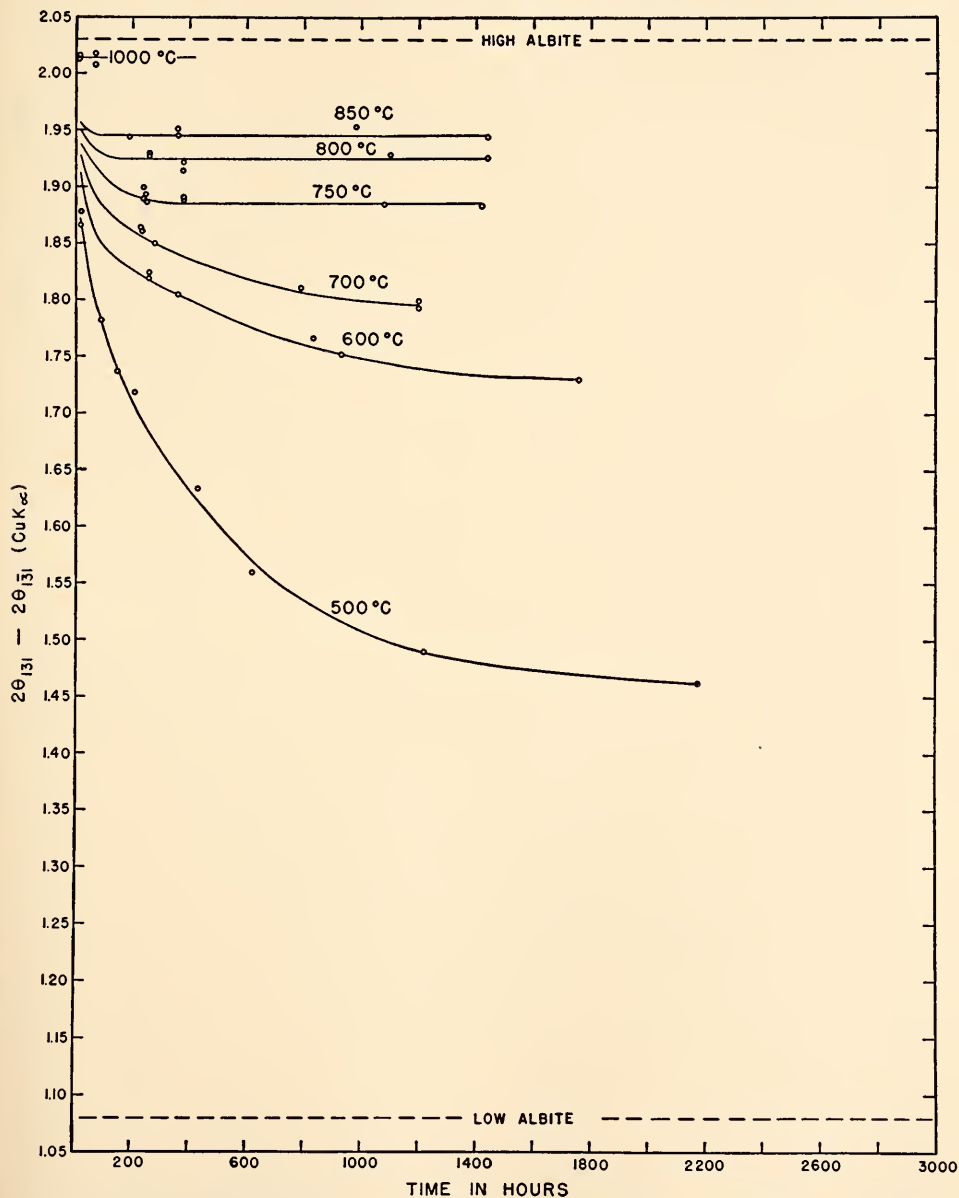


FIG. 23. Diagram showing the relation between temperature, time of crystallization, and  $2\Theta_{131} - 2\Theta_{1\bar{3}1}$  (CuK $\alpha$ ) for albites synthesized in the presence of water vapor from a glass of composition  $\text{NaAlSi}_3\text{O}_8$ .

was 14,000 pounds per square inch for all runs except those at 700° C, for which it was 28,000 psi, and those at 1000° C, for which it was 2500 psi. It has not been shown conclusively that in every case the crystals have exactly the composition  $\text{NaAlSi}_3\text{O}_8$ , since the composition of the vapor in the sealed platinum capsules has not been determined. If it is assumed that variations in composition of the crystals are negligible, the results of these experiments indicate that for each temperature there is a unique stable crystalline form of albite. As the crystallization temperature is lowered, a longer time is required to reach the equilibrium form for that temperature.

From this diagram it can be seen that crystals having a value of  $2\Theta_{131} - 2\Theta_{1\bar{3}1}$  more than halfway toward the low-temperature form of albite have been synthesized, and at a lower temperature it may be possible to synthesize material having physical properties almost identical with those of low-temperature albite. Experiments with this object in view are in progress.

By the criterion used here, the crystals formed at, say, 500° C are, after a few hours, similar to those formed at much higher temperatures and apparently at equilibrium at the higher temperatures. In some respects this finding may be compared with the observations which prompted Ostwald to propose the Law of Successive Reactions: i.e., the least stable condition obtains first and changes gradually to the more stable condition at the temperature considered. It should be noted, however, that Ostwald was considering phase changes in which two phases coexisted at the temperature of inversion. We are here concerned not with an abrupt change of phase at one temperature, but with a continuous change in the physical properties of the crystalline material over a large range in temperature.

Such a gradual change in properties over a range in temperature is what might be expected if the difference between the high-

temperature and low-temperature forms of albite is due to the difference in the distribution of the aluminum and silicon atoms, as has been postulated by a number of workers.

#### EFFECTS OF HEATING NATURAL PLAGIOCLASES

J. R. SMITH

It is known that sodic plagioclases in volcanic rocks which cooled quickly from magmatic temperatures differ structurally and optically from those which cooled very slowly from magmatic temperatures or formed at lower temperatures. For convenience the former are called "high-temperature" and the latter "low-temperature" plagioclases. Plagioclases synthesized in the dry way and those crystallized rapidly in the presence of water differ in still greater degree from low-temperature natural plagioclases, but they are included under the term "high-temperature plagioclases." Heating low-temperature plagioclases causes them to change sluggishly to the high-temperature forms; the accompanying changes in certain physical parameters of plagioclases are discussed in the following paragraphs.

The angular separation between the (131) and (1 $\bar{3}$ 1) reflections in X-ray powder diffraction patterns of sodic plagioclases changes markedly on heating the low-temperature forms; this fact affords a convenient means of determining the inversion state of a plagioclase. To investigate the rate and nature of the inversion in sodic plagioclases, several samples were held at constant temperatures, and fractions of the samples were quenched and X-rayed at intervals. It was found that  $2\Theta_{(131)} - 2\Theta_{(1\bar{3}1)}$  for Amelia albite changed from the original value, 1.06°, to 1.98° in 21 days at 1080° C, the rate of change decreasing markedly with time. No further change took place during subsequent heating for a total of 42 days. The starting material and the final product of the experiment gave sharp, well defined peaks in X-ray powder patterns, whereas the partly in-



verted material gave broad and, in some cases, double peaks. Under the microscope, the partly inverted grains were seen to have rims with different optical orientation from the cores. Some grains contain small blebs and spheres, which are probably glass formed by melting around submicroscopic inclusions. Albite from Ramona, California, behaves almost exactly like Amelia albite, but albite from Varuträsk, Sweden, changes much more slowly under the same conditions. In all the albite specimens investigated, the partly inverted material gave broad peaks in powder patterns.

$2\Theta(1\bar{3}1) - 2\Theta(1\bar{3}\bar{1})$  for Bakersville oligoclase ( $An_{21.6}$ ) changes from  $1.51^\circ$  to  $1.95^\circ$  in 35 days at  $1050^\circ\text{C}$ . Partly inverted material gave sharply resolved peaks in powder patterns, and there are no large differences in the optical orientations of cores and rims of the individual grains in the sample, such as were found in partly inverted albites. The difference between the inversion behavior of pure albites and Bakersville oligoclase suggests that, under the conditions of the experiment, the mechanism of the inversion in pure albite involves the nucleation and growth of a new phase, whereas the inversion in Bakersville oligoclase appears to be a continuous change in one phase, the material remaining homogeneous throughout the inversion.

The presence of water greatly accelerates the change of low-temperature sodic plagioclases to the high-temperature forms. For example,  $2\Theta(1\bar{3}1) - 2\Theta(1\bar{3}\bar{1})$  for Amelia albite changes from  $1.06^\circ$  to  $1.95^\circ$  in 13 days at  $1080^\circ\text{C}$  in air; in water at  $975^\circ\text{C}$  and 250 bars pressure, the same change takes place in 1 day. In experiments to investigate the lowest temperatures at which the change from low- to high-temperature forms will take place, it was found that two samples of oligoclase ( $An_{21.6}$  and  $An_{28.7}$ ) change measurably toward the high-temperature forms when held in water at  $690^\circ\text{C}$  and 30,000 psi for 2 weeks. There is no measurable change in albites under the same conditions. Although the changes involved are small in runs of this duration,

it may be concluded that the temperatures of stability of low-temperature calcic oligoclases are lower than  $690^\circ\text{C}$ .

The effects of heating on the optical properties of natural plagioclases are being investigated, and preliminary results are at hand. The samples were heated for 1 month in air at temperatures about  $30^\circ\text{C}$  below the solidus (at the respective compositions) in the binary system  $\text{NaAlSi}_3\text{O}_8 - \text{CaAl}_2\text{Si}_2\text{O}_8$ . Heating for shorter periods at lower temperatures resulted in a considerable range of  $2V$  from grain to grain in each sample, suggesting that the grains had changed by different amounts. In all the samples, numerous specks of glass formed in the grains, presumably around inclusions of foreign material. These specks of glass in the grains make refractive index measurements much less precise than in glass-free material. For this reason, samples will be inverted in the presence of water at lower temperatures, with the object of obtaining glass-free material for refractive index measurements. The specks of glass do not interfere with measurements of  $2V$  and birefringence, and the changes in these properties have been studied. Figure 24 shows a curve for  $2V$  of low-temperature natural plagioclases and a tentative curve for high-temperature sodic plagioclases. The large differences in  $2V$  between the high- and low-temperature sodic plagioclases agree with the data of Tuttle and Bowen, but the difference of about  $7^\circ$  between the  $2V$ 's of high- and low-temperature forms in the composition range  $An_{60}$  to  $An_{90}$  has not hitherto been reported. In part of this range, from  $An_{75}$  to  $An_{90}$ , the difference in  $2V$  may well be the only convenient means of distinguishing between the high- and low-temperature forms, because differences in X-ray powder patterns are very slight or absent, and single-crystal X-ray patterns are ambiguous. Changes in birefringence are analogous to the changes in  $2V$ , being greatest for albite ( $-0.002$ ), zero for  $An_{50}$ ,  $-0.001$  for  $An_{80}$ , and zero for anorthite. Preliminary measurements of refractive

index indicate that  $N_x$  changes slightly in albite, but negligibly for more calcic plagioclases, so that measurements of  $N_x$  may be used to determine the composition of a plagioclase regardless of its state of inversion.

$\text{CaAl}_2\text{Si}_2\text{O}_8$ . This chemical simplicity makes them amenable to experimental investigation and permits application of the results directly to natural rocks.

The chemical system outlined was first investigated in the dry way by determining

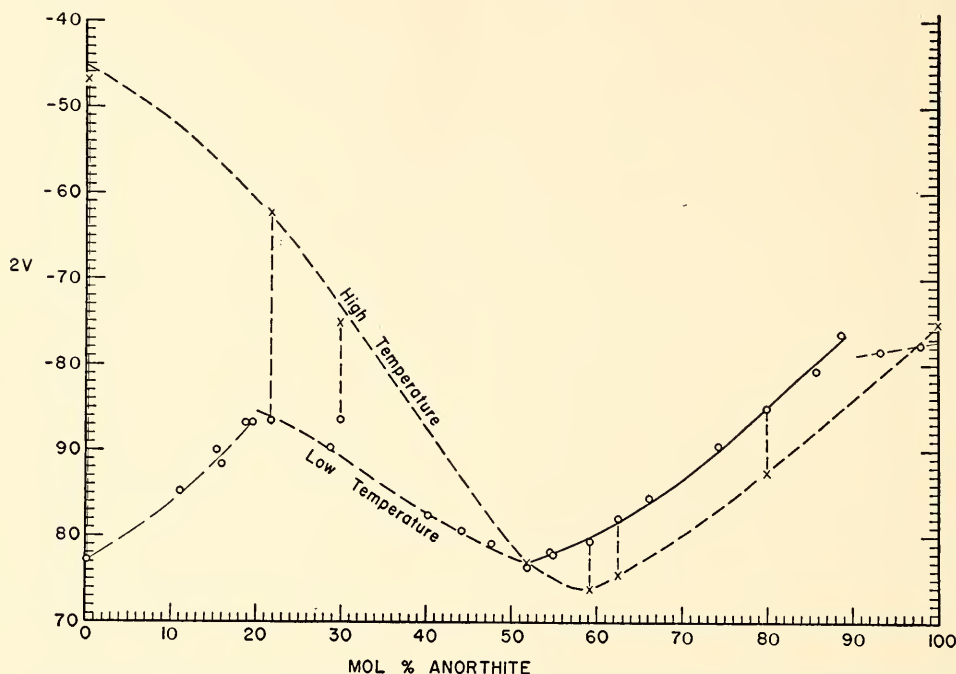


FIG. 24. Optic axial angles of low-temperature natural plagioclases (circles) and of some of the same samples after they had been heated at temperatures near the solidus (crosses). The cross at  $\text{An}_{100}$  represents the optic axial angle of anorthite synthesized in the dry way.

#### TERNARY FELDSPARS

H. S. YODER, JR., D. B. STEWART, AND  
J. R. SMITH

The feldspars are ubiquitous constituents of the igneous and metamorphic rocks and comprise almost 60 per cent of the earth's crust. Because of their extreme mineralogical complexity, they are not well understood; yet, as a consequence of their complexity, they will be most valuable as a record of the environmental conditions at the time of growth. In addition, the feldspars are unique in that they can be represented almost entirely by a relatively simple chemical system having as components  $\text{NaAlSi}_3\text{O}_8$ ,  $\text{KAlSi}_3\text{O}_8$ , and

the melting relations of each of the end-member feldspars (Day and Allen, 1905; Greig and Barth, 1938; Morey and Bowen, 1922); each pair of feldspars (Bowen, 1913; Schairer, 1950; Schairer and Bowen, 1947); and finally the ternary feldspars (Franco and Schairer, 1951). These studies fixed the liquidus relations; because of the difficulty of obtaining equilibrium, however, it was not possible to define the solidus accurately, and impracticable to investigate the subsolidus region. In order to solve the problem of attaining equilibrium, each of the feldspars was in turn investigated in the presence of water under pressure. The  $\text{NaAlSi}_3\text{O}_8\text{—H}_2\text{O}$  system was first

studied by Goranson (1938), later by Yoder (unpublished), and by Tuttle and Bowen (unpublished). The  $\text{KAlSi}_3\text{O}_8\text{—H}_2\text{O}$  system was investigated by Goranson (1938) and by Yoder, Stewart, and J. R. Smith (unpublished), and the  $\text{CaAl}_2\text{Si}_2\text{O}_8\text{—H}_2\text{O}$  system by Yoder (unpublished). The first of the feldspar pairs to be studied in the presence of water,  $\text{KAlSi}_3\text{O}_8\text{—NaAlSi}_3\text{O}_8\text{—H}_2\text{O}$ , was successfully outlined by Bowen and Tuttle (1950). There remained to be studied the feldspar pairs in the systems  $\text{KAlSi}_3\text{O}_8\text{—CaAl}_2\text{Si}_2\text{O}_8\text{—H}_2\text{O}$  and  $\text{NaAlSi}_3\text{O}_8\text{—CaAl}_2\text{Si}_2\text{O}_8\text{—H}_2\text{O}$ , as well as the ternary feldspars in the system  $\text{KAlSi}_3\text{O}_8\text{—NaAlSi}_3\text{O}_8\text{—CaAl}_2\text{Si}_2\text{O}_8\text{—H}_2\text{O}$ . This task has been undertaken by Yoder, Stewart, and Smith, and the preliminary diagrams for the bounding systems of the ternary feldspars in the presence of water are already taking form.

From previous experience it was known that equilibrium could be obtained most readily at the highest water pressure in the required temperature range. Because of the numerous compositions to be studied, it was necessary to select a single high pressure at which all the equilibrium relations would be determinable in a reasonable amount of time. The choice of 5000 bars was based on the fact that this pressure is sufficiently high to approach equilibrium in a relatively short time and yet is conveniently attainable in Yoder's apparatus even at the high temperatures needed ( $1250^\circ\text{C}$ ), and on the belief that some of the complexities of the melting relations would be alleviated by the known effects of water on the liquidus surface. It will be necessary, of course, to investigate other pressures after the relations for 5000 bars are known. The results of the present studies are presented in the preliminary diagrams given in figures 25, 26, and 27.

In figure 25 is shown the projection of the system  $\text{NaAlSi}_3\text{O}_8\text{—KAlSi}_3\text{O}_8\text{—H}_2\text{O}$  for 5000 bars. It must be kept in mind that the liquids in this and the following systems to be described are saturated with water to the extent of 9 to 12 weight per

cent and are in equilibrium with an aqueous fluid phase, and therefore the nomenclature will pertain to such a ternary system. Bowen and Tuttle (1950) previously outlined the system at approximately 1000 and 2000 bars water pressure. As found by Bowen and Tuttle, the phases obtained belong to the high albite—high sanidine series. The most significant change is that the leucite field is absent. The point on the three-phase trough, the lowest temperature on the projected liquidus in figure 25, lies at  $695^\circ\text{C}$ , and the composition of the solid phase is in the ratio of 29  $\text{KAlSi}_3\text{O}_8$  to 71  $\text{NaAlSi}_3\text{O}_8$ . Although the solvus as determined by Bowen and Tuttle at about 1000 bars may not be identical with that at 5000 bars, it is within  $35^\circ$  of the point on the three-phase trough. Pressure affects the solvus, but the magnitude of the effect is probably small. There is some uncertainty as to the exact composition and temperature of the maximum and the character of the solvus near the maximum. In the present work, equilibrium in the subsolidus region was still difficult to attain; runs of 5 days' duration at the high pressure failed to produce unequivocal results. On the basis of the short runs made, it is estimated that several weeks or more will be required to attain equilibrium at the low temperatures of the subsolidus region even at the high water pressure. As will be seen below, it is of paramount importance that the solvus be fixed with considerable accuracy.

In figure 26 is given the projection of the ternary system  $\text{NaAlSi}_3\text{O}_8\text{—CaAl}_2\text{Si}_2\text{O}_8\text{—H}_2\text{O}$  for 5000 bars. The shape of the region where liquid + crystals are stable is essentially that determined by Bowen (1913) in the dry way; the liquidus temperatures are depressed over  $300^\circ\text{C}$  by the addition of water at 5000 bars, however. As in most systems, the solidus is difficult to fix, and only one reliable bracket has been made so far in the present work. Aside from this difficulty, a "β-alumina" of unknown composition has appeared in runs at and near the  $\text{CaAl}_2\text{Si}_2\text{O}_8$  composi-

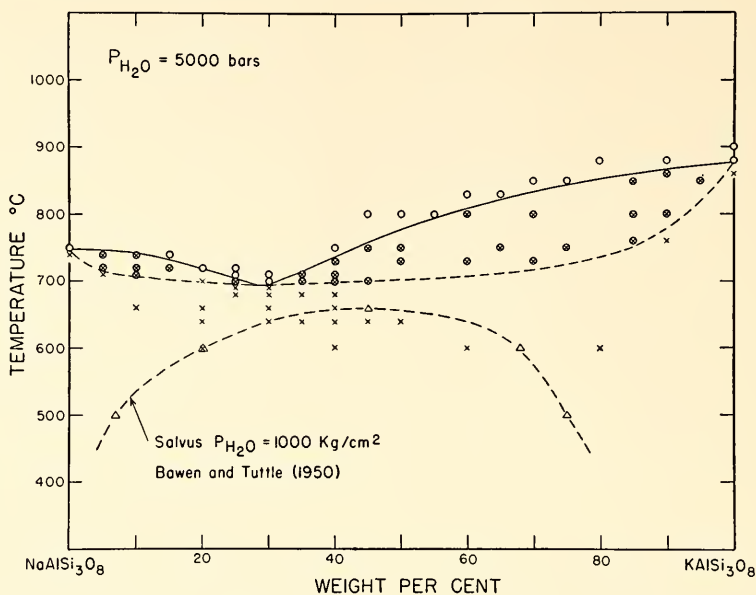


FIG. 25. Preliminary projection of the ternary system  $\text{NaAlSi}_3\text{O}_8$ — $\text{KAlSi}_3\text{O}_8$ — $\text{H}_2\text{O}$  at 5000 bars water pressure. The solvus (dashed curve marked by triangular points) is that determined by Bowen and Tuttle (1950) at about 1000 bars water pressure.

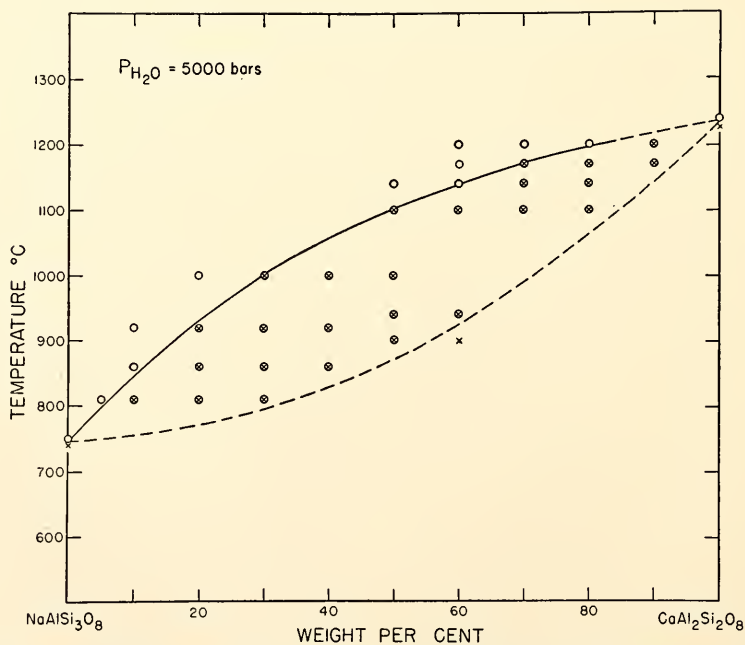


FIG. 26. Preliminary projection of the ternary system  $\text{NaAlSi}_3\text{O}_8$ — $\text{CaAl}_2\text{Si}_2\text{O}_8$ — $\text{H}_2\text{O}$  at 5000 bars water pressure.



tion. It is most likely that the " $\beta$ -alumina" is a metastable phase and will be resorbed with longer runs. On the other hand, the " $\beta$ -alumina" may be converted to corundum. On the anhydrous join  $\text{CaAl}_2\text{Si}_2\text{O}_8$ — $\text{Al}_2\text{O}_3$  (Rankin and Wright), the boundary of the corundum field lies close to the anorthite composition. This observation, in addition to the probably larger effect of  $\text{H}_2\text{O}$  on the liquidus surface of anorthite, sug-

ternary if anorthite melts congruently. The principal points of interest are that the maximum amount of  $\text{CaAl}_2\text{Si}_2\text{O}_8$  in solid solution with  $\text{KAlSi}_3\text{O}_8$  is less than 3 weight per cent and the maximum amount of  $\text{KAlSi}_3\text{O}_8$  in solid solution with  $\text{CaAl}_2\text{Si}_2\text{O}_8$  is less than 5 weight per cent for this specific isobaric section. These values have important bearing on the extent of the solid solutions in the ternary feldspar

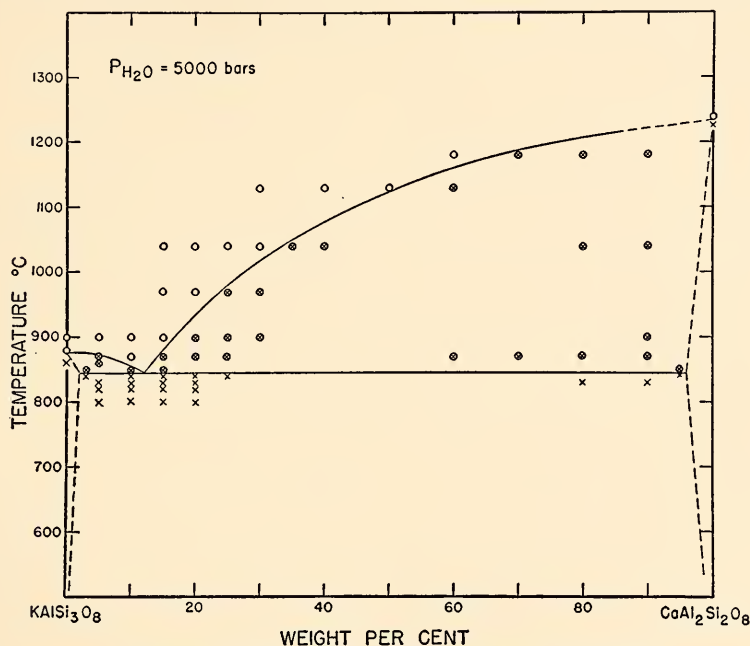


FIG. 27. Preliminary projection of the ternary system  $\text{KAlSi}_3\text{O}_8$ — $\text{CaAl}_2\text{Si}_2\text{O}_8$ — $\text{H}_2\text{O}$  at 5000 bars water pressure.

gests that anorthite may be melting incongruently to corundum+liquid under high water pressures. Hexagonal  $\text{CaAl}_2\text{Si}_2\text{O}_8$  has appeared in a few runs and is presumed to be metastable. The presence of quartz in some runs suggests that the composition of vapor in these cases did not lie in the volume under investigation.

The projection of the  $\text{KAlSi}_3\text{O}_8$ — $\text{CaAl}_2\text{Si}_2\text{O}_8$ — $\text{H}_2\text{O}$  system at 5000 bars is given in figure 27. The complexities arising from the leucite field in the anhydrous system are now absent, and the system is wholly

system. The point on the four-phase boundary curve is at about  $845^\circ\text{C}$ , and the composition of the solid phases is in the ratio of 12  $\text{CaAl}_2\text{Si}_2\text{O}_8$  to 88  $\text{KAlSi}_3\text{O}_8$  by weight.

From these preliminary diagrams it is possible, of course, to construct an estimate of the quaternary system  $\text{KAlSi}_3\text{O}_8$ — $\text{NaAlSi}_3\text{O}_8$ — $\text{CaAl}_2\text{Si}_2\text{O}_8$ — $\text{H}_2\text{O}$  in which some of the phase relations of the natural ternary feldspars are readily recognized. From this construction it is clear that the principal problem will be the determina-

tion of the composition of the ternary feldspars. The geometry of the various crystal+liquid surfaces is sufficiently distinctive for the maximum solid solution to be ascertained; new optical and X-ray methods will be required, however, to fix the extent of the solid solution at subsolidus temperatures. To investigate this particular facet of the problem, isothermal sections at 5000 bars will be made and the phase boundaries will be determined.

The most critical region of the system with regard to natural feldspars is dependent on the manner in which the solvus, which rises from the  $\text{NaAlSi}_3\text{O}_8$ — $\text{KAlSi}_3\text{O}_8$  join, intersects the solidus of the four-component system. In view of the small amount of solid solution of  $\text{CaAl}_2\text{Si}_2\text{O}_8$  in  $\text{KAlSi}_3\text{O}_8$  obtained experimentally and the known compositions of natural alkali feldspars, it is likely that the first contact of the solvus and solidus will take place close to the  $\text{NaAlSi}_3\text{O}_8$ — $\text{KAlSi}_3\text{O}_8$  join. The point of contact of the solvus with the liquid+crystals region may take place at or on either side of the critical point (the point where the feldspars in the two-feldspar region are indistinguishable). This critical point must lie outside the trough of minimum temperature on the liquidus surface, in view of the quaternary nature of the crystal+liquid region. These intricate phenomena are expected to take place within a narrow temperature and composition interval, and their definition may be difficult experimentally.

The study of the ternary feldspars in the presence of water is a prerequisite to understanding both the equilibrium and the nonequilibrium relations in the natural feldspars. Foremost of the equilibrium processes is the course of crystallization as a function of temperature and pressure. Since the feldspars are the principal mineral group in the igneous rocks, this knowledge will be an important tool in deciphering the genesis of these rocks. The determination of the properties of synthetic ternary feldspars of known composition will be most useful in the determination of the composition of the natural feldspars.

In addition, knowledge of the composition of feldspars in equilibrium with one another may serve as the basis for a geothermometer. Among the nonequilibrium processes, the course of fractionation may be read from the equilibrium diagrams, and such information will aid in the understanding of rock assemblages. Also, some of the zoning and mantling relations (see Rapakivi Texture, below) among the feldspars may be elucidated. There is little doubt that the ternary feldspar system is the most important mineral system in petrology.

### RAPAKIVI TEXTURE

D. B. STEWART

Rapakivi texture, alkali feldspar mantled by plagioclase, has been an unresolved problem in petrography for sixty years. The rapakivi feldspars were examined in a study begun in 1951 at Harvard University to see whether their properties were those predicted by the phase diagrams available for the system  $\text{Or}$ — $\text{Ab}$ — $\text{SiO}_2$ — $\text{H}_2\text{O}$ .

Accordingly, the composition and polymorphic forms were determined for feldspars from both varieties of coarse-grained rapakivi granite from a batholith in eastern Penobscot Bay, Maine. A magmatic origin for the granite was indicated by the intrusive nature of its contact and by the oriented minerals and xenoliths it contained. At most localities microcline from the cores of rapakivi ovoids, from coexisting phenocrysts without mantles, from the groundmass, and porphyroblasts from inclusions had the same bulk composition ( $\text{Or}=69$  mol per cent) within the error of measurement ( $\pm 3$  per cent  $\text{Or}$ ). Plagioclase of the low form in the rock at the same localities varied from  $\text{An}_3$  to  $\text{An}_{36}$ ; the most common composition of the rapakivi mantle was  $\text{An}_{22}$ . Chemical analyses were made of oligoclase ( $\text{Ab}_{73.0}\text{An}_{24.2}\text{Or}_{2.8}$ ) from rapakivi mantles and microclines ( $\text{Or}_{70.6}\text{Ab}_{27.5}\text{An}_{1.9}$ ) from the core adjacent to the mantle, and of the granite containing the ovoids. No rapakivi ovoids formed in the contact-metamorphosed

country rock; the progressive stages of reaction leading to the development of rapakivi ovoids are, however, preserved in xenoliths of country rock.

These observations indicated that the additional component An must be considered. In the light of phase diagrams deduced for the system Or—Ab—An—SiO<sub>2</sub>—H<sub>2</sub>O, the rapakivi texture can be explained as a product of magmatic processes. A quantitative study of various portions of this system is under way at present at this Laboratory (Yoder, Stewart, J. R. Smith).

#### ALKALI FELDSPARS IN IGNEOUS ROCKS

W. S. MACKENZIE AND J. V. SMITH

This project, which was begun in 1954 by MacKenzie and J. V. Smith, has been continued over the past two years although Smith is now at Cambridge University in England. Well mapped rock masses were selected, and the alkali feldspar was studied by optical and X-ray methods.

The feldspars from the Tardree rhyolite, County Antrim, are sanidine cryptoperthites in which only very slight unmixing can be detected. In the porphyritic felsite of Slieve Gullion, the feldspars range from sanidine cryptoperthites to orthoclase microperthites; those from the granophyre of Slieve Gullion range from sanidine cryptoperthites to orthoclase-microcline perthites. The Beinn an Dubhaich granite contains sanidine-orthoclase perthites, whereas the Arran and Mourne granites contain only orthoclase perthites. The alkali feldspars of the Dartmoor granite are orthoclase-microcline perthites. On the basis of geological evidence there appears to be some correlation between the type of alkali feldspars developed and an estimated rate of cooling of the rock masses. It is unlikely, however, that this is the only factor involved, and doubtless the presence or absence of volatiles is of considerable importance in determining the state of the feldspars.

There seems to be little doubt that a microcline—low albite perthite may develop in the later stages of the slow cooling

of a rock mass as a result of unmixing of a single-phase high-temperature feldspar, since all intermediate stages in this process have been found. It is hoped that some criteria may emerge from this work which will enable a distinction to be made between a perthitic intergrowth in which two feldspars have unmixed from a single high-temperature feldspar, on the one hand, and an intergrowth which has resulted from partial replacement of one feldspar by another. The textures of string and film perthites, as seen under the microscope, are doubtless the result of an unmixing process, but some of the coarser perthites may be the products of replacement. This is a most important question, since the petrologist may be strongly influenced by textural evidence in attempting to reconstruct the history of a particular rock mass, and in plutonic rocks such evidence may be very misleading.

#### REFRACTIVE INDICES OF PLAGIOCLASE GLASSES

J. F. SCHAIRER, J. R. SMITH, AND F. CHAYES

Refractive indices of plagioclase glasses made by Schairer have been measured with an estimated accuracy of  $\pm 0.001$ , for the benefit of those who wish to determine the compositions of plagioclases by melting them and measuring the refractive indices of the glasses. The data are shown in figure 28 and in the first two columns of table 12. They are in substantial agreement with measurements published by Larsen in 1909, but are somewhat more reliable because the chemical constituents of the glasses can be obtained in purer form today, and a greater number of samples were available to the writers.

The index of refraction and the weight per cent of CaAl<sub>2</sub>Si<sub>2</sub>O<sub>8</sub> in each glass are shown in the first two columns of table 12. The third column shows the estimate of composition given by the best-fitting straight line, the formula of which is

$$\hat{Y}_e = 1139.444(n - 1.4) - 97.414, \quad (1)$$

where  $n$ , the index of refraction, is taken as independent variable, and  $\hat{Y}_e$  is the estimated composition. The fourth column



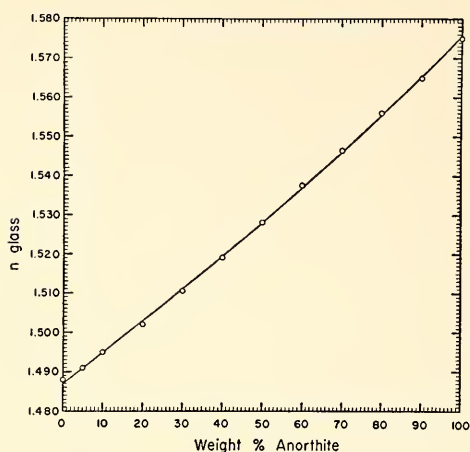


FIG. 28. Indices of refraction of glasses of plagioclase compositions. The curve is the best-fitting parabola of equation (2), text.

of the table is a similar set of estimates obtained from the best-fitting parabola,

$$\hat{Y}_p = 1653.782(n - 1.4) - 1987.25(n - 1.4)^2 - 129.036. \quad (2)$$

It will be noted that the first three entries in column 3 are larger than their equivalents in column 2, the next six are smaller, and the last three are again larger. The parabola was fitted largely because of this

TABLE 12

INDICES OF REFRACTION AND COMPOSITIONS OF SOME GLASSES OF PLAGIOCLASE COMPOSITION

$n$	$Y$	$\hat{Y}_e$	$\hat{Y}_p$
1.488	0	2.86	1.11
1.491	5	6.28	5.00
1.495	10	10.83	10.14
1.502	20	18.81	18.97
1.510 <sub>5</sub>	30	27.92	29.44
1.519	40	38.18	39.62
1.528	50	48.44	50.09
1.537 <sub>5</sub>	60	59.33	60.79
1.546 <sub>5</sub>	70	68.94	70.59
1.556	80	80.34	80.59
1.565	90	90.59	89.73
1.575	100	101.99	99.52

$n$ , index of refraction;  $Y$ , chemical composition, weight per cent  $\text{CaAl}_2\text{Si}_2\text{O}_8$ ;  $\hat{Y}_e$ , composition predicted from equation (1);  $\hat{Y}_p$ , composition predicted from equation (2).

strongly systematic trend in the deviations from linear regression. Introduction of a higher-order term does materially reduce the tendency toward systematic discrepancies. For 10 of the 12 glasses it has the further advantage of yielding deviations that are also smaller in absolute size. This is perhaps best summarized by the calculated errors of estimate, 1.66 per cent  $\text{CaAl}_2\text{Si}_2\text{O}_8$  for predictions based on equation (1), 0.70 per cent for those based on equation (2).

Two more general problems involved in work of this type may be briefly discussed here. A better fit than is given by the single straight line could also have been obtained by fitting separate straight lines to the upper and lower thirds of the range and extrapolating inward, as has been done for the  $\gamma$  index of natural plagioclase (Year Book No. 49, 1949-1950, p. 53). If such paired lines provide a considerably better fit than a single full-range line, it is sometimes taken as evidence for a physical discontinuity in the parameter of which the measurements are estimates. Unless the paired lines are also a considerable improvement over the appropriate parabola or other higher-order curve, however, the argument is difficult to support. If independent evidence demonstrates, or even strongly suggests, the existence of a discontinuity, the paired-line technique is often a legitimate device for locating it. Present knowledge of the structure of glasses of plagioclase composition offers no reason to suppose the existence of a discontinuity in the rate of change of composition with index of refraction.

Finally, it is often urged that the mol per cent is a more "natural" unit for calculations of this sort, and in support of this preference it is pointed out that relations that are strictly linear in either form of statement will be curvilinear in the other. In practice, however, a sizable array of observations whose variation is strictly linear in *either* form of statement is almost never encountered. The present case is an excellent example; departure from linearity is quite as noticeable and quite as system-



atic whether composition is stated in mol per cent or in weight per cent.

As a preliminary test of the usefulness of the data for determinative purposes, five samples of chemically analyzed natural plagioclases (two from pegmatites and three from layered intrusions) were melted, and the refractive indices of the glasses were measured. The compositions of the samples predicted by equation (2) differ by less than 1.5 per cent anorthite from the compositions as given by the chemical analyses if the analyses are recalculated as  $[An/(An + Ab + Or)] \times 100$ , using only the determinations of lime and alkalis.

#### THE SYSTEM $NaAlSi_3O_8-KAlSi_3O_8-SiO_2-H_2O$

W. S. MacKENZIE

Under the stimulus of a visit by Professor C. E. Tilley to the Laboratory, some preliminary experiments in the silica-poor part of petrogeny's residua system were made, the object being to determine the direction of the tie lines joining the compositions of coexisting nephelines and feldspars for given bulk compositions at different temperatures.

A glass of composition  $Qtz_{35}Ne_{45}Kp_{20}$ , which approximates the composition of lardalites, was crystallized in the presence

of water vapor at a pressure of 8000 psi at temperatures from 800° to 500° C. At 800° C a small amount of glass was formed, with nepheline and feldspar. Between 775° and 625° C the compositions of the feldspar and nepheline did not change within the limits of error in the measurement of the compositions from X-ray diffraction patterns; these compositions were determined to be  $Or_{46}Ab_{54}$  and  $Ne_{93}Kp_7$ , respectively. (The  $SiO_2$  content of a nepheline cannot be determined from the X-ray diffraction pattern.) At 500° C analcite and a potassium-rich feldspar are formed.

The formation of analcite at 500° C limits very considerably the temperature range over which this experimental work can be carried out, and it would appear that for this composition a much greater range of temperature will have to be covered before changes in the direction of the tie lines can be observed. By working at much lower water vapor pressures it will be possible to lower the temperature at which analcite first appears. A number of glasses corresponding approximately in composition to other types of nepheline syenites were crystallized at one temperature, 600° C, and in this way a preliminary isothermal section of the silica-poor part of the system has been drawn up.

#### MICAS

H. P. EUGSTER

*Muscovite-paragonite assemblages.* The equilibrium diagram for the join muscovite-paragonite in the subsolidus region was published in Year Book No. 54. In this diagram the composition of the paragonites had been inferred from natural muscovite-paragonite assemblages. Runs have since been made to determine the composition of paragonite in equilibrium with muscovite up to 650° C on synthetic material. The final diagram is now available. Recently Grootemaat and Holland at Princeton University used this diagram to determine the temperature of formation of muscovite-albite assemblages of different zones

in the Peerless pegmatite (Black Hills, South Dakota). The information obtained has been valuable for explanations of the emplacement of large zoned pegmatites. The work is being expanded to include other pegmatites and pegmatite-like bodies in the Black Hills.

*Phlogopite.* In the hope that the soda content of phlogopite from phlogopite-albite assemblages can also be used as a geothermometer, the soda content of synthetic phlogopite in equilibrium with albite is now being determined up to 900° C. This thermometer will have a wider application, and it is expected that it can be

modified to include biotite-albite assemblages.

**Biotite.** The work on the stability of annite has been reported above (see p. 158). All natural biotites contain some magnesium. Knowledge of the phase relations on the join phlogopite-annite is therefore of

great geological importance. Work has been started to extend the data obtained for the end members to include all biotites. The investigations are complicated by the fact that for this system too the partial oxygen pressure must be considered as an independent variable.

## AMPHIBOLES

F. R. BOYD, JR.

Hydrothermal study of the calciferous amphibole group, initiated two years ago, has been bringing increased returns. This study has been concentrated largely on tremolite [ $\text{Ca}_2\text{Mg}_5\text{Si}_8\text{O}_{22}(\text{OH})_2$ ] and pargasite [ $\text{NaCa}_2\text{Mg}_4\text{Al}^{\text{VI}}\text{Al}_2^{\text{IV}}\text{Si}_6\text{O}_{22}(\text{OH})_2$ ], the two most important magnesian end members in this group. Progress this year has resulted principally in the completion of the stability studies on pure pargasite composition and in the collection of data on the join quartz-pargasite. Some X-ray and optical properties of synthetic pargasite and tremolite were included in last year's report and are not repeated here.

### PARGASITE

The  $T$ - $P_{\text{H}_2\text{O}}$  diagram for the magnesian hornblende pargasite [ $\text{NaCa}_2\text{Mg}_4\text{Al}^{\text{VI}}\text{Al}_2^{\text{IV}}\text{Si}_6\text{O}_{22}(\text{OH})_2$ ] is presented in figure 29. This diagram consists of three phase boundaries (curves *A*, *B*, and *C*) which intersect at an invariant point (*d*). Boundary *A* is the curve for the breakdown of pargasite to anhydrous crystalline phases + vapor. Boundary *B* represents the beginning of melting of these anhydrous phases in the presence of vapor. The stable phase assemblage in the  $T$ - $P_{\text{H}_2\text{O}}$  region bounded by curves *A* and *B* consists of aluminous diopside + nepheline + forsterite, minor amounts of spinel and anorthite, and vapor. If this crystalline phase assemblage is placed in the  $T$ - $P_{\text{H}_2\text{O}}$  region above curve *B*, it undergoes partial melting and the stable phases become aluminous diopside + forsterite + spinel + liquid + vapor. Curves *A* and *B* intersect at an invariant point at 1025° C and 800 bars ( $\text{H}_2\text{O}$ ). At greater

pressures, pargasite heated at constant  $\text{H}_2\text{O}$  pressure no longer breaks down in the solid state, but melts incongruently to aluminous diopside + forsterite + spinel + liquid + vapor. The curve *C*, along which incongruent melting of pargasite takes place, has been traced from the invariant point (*d*) out to a pressure of 1200 bars ( $\text{H}_2\text{O}$ ).

The stability field of pargasite, bounded on the high-temperature side by curves *A* and *C*, extends to higher temperatures than any other investigated hydrous silicate except the magnesian mica phlogopite. At 1000 bars ( $\text{H}_2\text{O}$ ) the stability limit of the amphibole pargasite is intersected at 1045° C. At 1000 bars ( $\text{H}_2\text{O}$ ) the stability field of the mica phlogopite, as determined by Yoder and Eugster, extends to this same temperature. The coincidence of the stability ranges of pargasite and phlogopite seems to reflect the *relative* stabilities of hornblendes and biotites in more complex natural systems. The mineral assemblages augite + hypersthene + plagioclase and hypersthene + potash feldspar, which are the anhydrous chemical equivalents of, respectively, hornblende + quartz and biotite + quartz, make their appearance in the granulite facies at roughly the same metamorphic grade.

Perhaps the most interesting aspect of the investigation of pargasite is the manner in which the diagram in figure 29 reflects the relations among certain mineral facies in metamorphic and igneous rocks. Hornblendes commonly are found in plutonic rocks and as phenocrysts in lavas. The

physical conditions under which hornblende-bearing magmatic rocks have formed are such that, if these conditions were to be projected on a  $T$ - $P_{H_2O}$  plane, the fields comprising the amphibolite facies and the magmatic facies must directly adjoin one another. There are metamorphic rocks, nevertheless, whose bulk composi-

pargasite corresponds to the amphibolite facies. In the region above boundaries  $B$  and  $C$  a liquid phase is stable, and this region corresponds to a magmatic facies. These two facies are in contact along curve  $C$ . Along this boundary, hornblende (in this system, pargasite) is stable in association with a melt, reflecting the occurrence

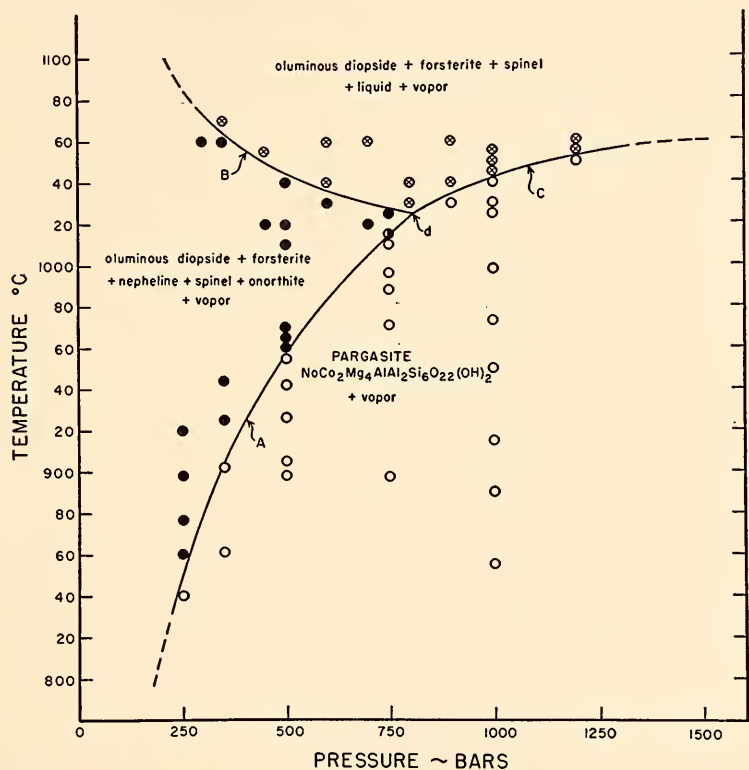


FIG. 29. The stability field of the magnesian hornblende pargasite

tions are such that they could contain hornblende, but which are in fact made up of anhydrous phases. These rocks are usually grouped in either the pyroxene hornfels or the pyroxene granulite facies. On a  $T$ - $P_{H_2O}$  projection, these two pyroxene facies must lie in a position intermediate between the amphibolite facies and the magmatic facies.

How these various facies may be arranged on a  $T$ - $P_{H_2O}$  diagram can be indicated by reference to the pargasite diagram in figure 29. Here the stability field of

of hornblendes as phenocrysts in lavas. In the  $T$ - $P_{H_2O}$  field bounded by curves  $A$  and  $B$ , neither an amphibole nor a melt can exist as a stable phase. This region corresponds to the pyroxene hornfels or pyroxene granulite facies; the distinction between them is probably to be sought in a difference of rock pressure not shown on the diagram. Thus the pyroxene facies represent conditions of low vapor pressure. In this particular system the vapor pressure must be less than that of the invariant



point (*d*) (800 bars  $\text{H}_2\text{O}$ ) for a subsolidus pyroxene assemblage to be stable at all.

#### PARGASITE-QUARTZ

The presence among the breakdown products of pargasite of strongly undersaturated phases such as forsterite and nepheline indicates that addition of quartz to the pargasite composition will markedly affect the amphibole boundary curve. Study of the join pargasite-quartz has therefore been undertaken. This join has turned out to be complex, and work on it has not yet been completed. Accordingly, only a preliminary view of some major points will be given at this time.

If an amount of silica in excess of that required to saturate all the pargasite breakdown products is added to the pargasite composition, the phase assemblage encountered above about  $900^\circ\text{C}$  at 1000 bars ( $\text{H}_2\text{O}$ ) is diopside + enstatite + labradorite + quartz. Below about  $900^\circ\text{C}$  at 1000 bars ( $\text{H}_2\text{O}$ ) an amphibole is obtained, but it is not pargasite. Its optical and X-ray properties indicate that it is close to tremolite in composition. Pargasite thus appears to have no stability field in the presence of an excess of silica. The stable assemblage on the join pargasite-quartz, within the stability field of amphibole and for compo-

sitions in which quartz appears as a phase, is an amphibole close to tremolite in composition + plagioclase + quartz.

At first these results seemed surprising, inasmuch as hornblendes, not far in composition from pargasite, occur naturally in association with quartz. If naturally occurring calciferous amphiboles very poor in iron are examined, however, the experimental observations prove to be in good agreement with the field data.

All the analyzed natural magnesian pargasites known to the author come from undersaturated environments. This relation is well shown at the type locality at Pargas, where quartz and pargasite have been noted to be incompatible phases. Moreover, a number of magnesian, calciferous amphiboles from high-grade, regional metamorphic assemblages, which include plagioclase and quartz, have been checked optically and found to be near tremolite in composition.

If, however, the occurrence in nature of iron-rich pargasites is examined, it is found that they are typically present in pegmatites and granites in association with quartz. The data from natural assemblages suggest that the Fe/Mg ratio in a silica-saturated rock may exercise a powerful control on the amount of soda and alumina an amphibole growing in such a rock can absorb.

#### CHLORITOID

L. B. HALFERDAHL

Chloritoid,  $\text{Fe}_2\text{Al}_4\text{Si}_2\text{O}_{10}(\text{OH})_4$ , a mineral thought by many geologists to be comparatively rare and to form only under very restricted conditions, has in fact been found at more than 70 localities. These occurrences include metamorphosed sedimentary rocks high in  $\text{FeO}$  and  $\text{Al}_2\text{O}_3$  and low in  $\text{CaO}$ , and hydrothermally altered rocks, many of which are lavas containing ore deposits. Consideration of the mineral assemblages in the many world-wide occurrences led to the hypothesis that chloritoid should form under a wide range of temperature and pressure conditions. In order

to test this hypothesis and to clarify the relation of chloritoid to staurolite, cordierite (fig. 30), and other common minerals with which it occurs, hydrothermal and petrologic investigations are being undertaken. In addition, because of the confusion in the literature regarding the physical properties and the chemical composition of chloritoid, X-ray, optical, and chemical studies are being carried out.

Samples of natural chloritoid,  $[(\text{Fe}''', \text{Mg}, \text{Mn})_2 (\text{Al}, \text{Fe}'')] (\text{OH})_4 \text{Al}_3 [\text{O}_2(\text{SiO}_4)_2]$  (structural formula proposed by Brindley and Harrison, 1952), have been obtained



from more than 40 localities. Powder X-ray diffraction patterns of many of these samples show that there are at least two distinct types, probably corresponding to the monoclinic and triclinic polymorphs reported by other workers on the basis of careful optical studies.

Figure 31 shows the chemical composi-

chemical analysis. Ideally, chloritoid should plot as a point on the dashed line. Deviations from this line toward  $\text{Al} + \text{Fe}''' + \text{Ti}$  are probably due partly to impurities such as magnetite, ilmenite, and rutile in the analyzed material and partly to partial oxidation of  $\text{Fe}''$ . Five new chemical analyses are being obtained.

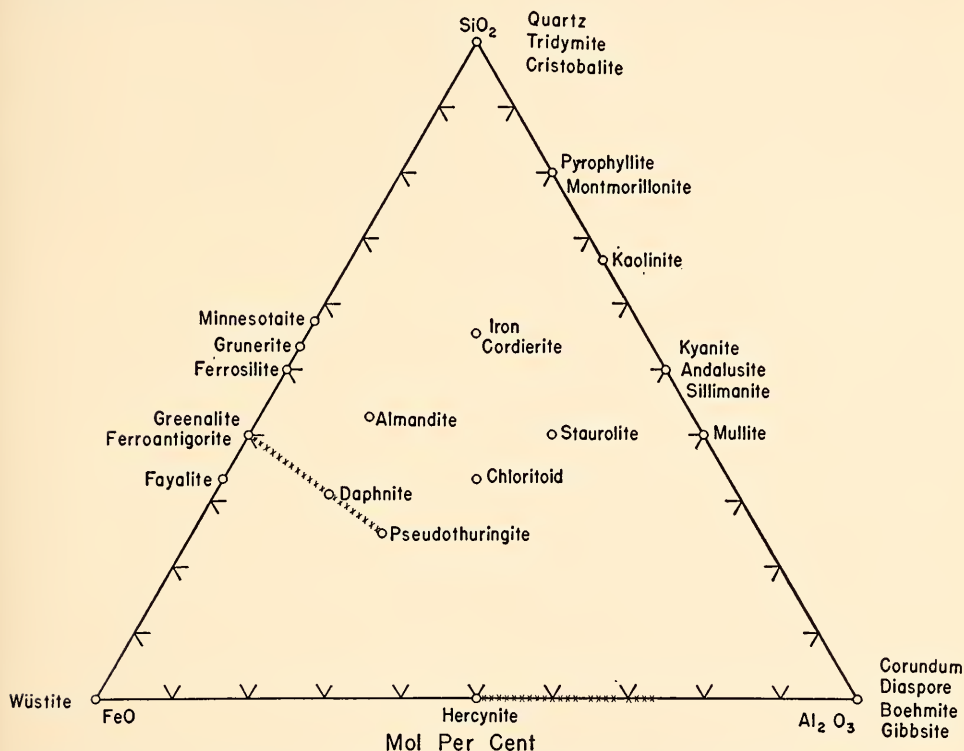


FIG. 30. Chemical relation of chloritoid to the other phases in the system  $\text{FeO}-\text{Al}_2\text{O}_3-\text{SiO}_2-\text{H}_2\text{O}$ . Hydrus phases have been projected onto the plane  $\text{FeO}-\text{Al}_2\text{O}_3-\text{SiO}_2$ . The lines of X's represent solid solution. The extent of the hercynite-corundum solid solution is not known.

tions of natural chloritoids on the basis of analyses selected from those available. Each triangle represents one chemical analysis. The corners of the triangles are the number of atoms in the mixed octahedral layer obtained by calculations on an anhydrous basis of 12 oxygen atoms per formula. The size of each triangle indicates the difference between the ideal number of atoms per formula in the mixed layer, 3, and the number obtained from the corresponding

Chloritoid has been synthesized at a total pressure of 10,000 bars up to  $675^\circ \pm 25^\circ \text{C}$ , from three starting mixtures of the requisite composition. Above this temperature at 10,000 bars, the crystalline products formed are staurolite<sup>4</sup> + quartz + hercynite-magnetite solid solutions; these products are also formed by the breakdown of natural

<sup>4</sup> After the failure to synthesize staurolite reported two years ago by Yoder, success has now been attained.

chloritoid under the same conditions. Because of the sluggishness of reaction and the difficulty of controlling the state of oxidation, however, these products may not represent equilibrium. At 2000 bars total pressure, natural chloritoid breaks down to iron cordierite + hercynite + vapor at temperatures at least as low as 600° C. The assemblage of iron cordierite and hercynite is

cordierite is stable only at low pressures. These observations are supported in nature by the common occurrence of cordierite in contact aureoles, whereas staurolite, not cordierite, is generally believed to form only in regionally metamorphosed terranes. At 2000 bars total pressure and at temperatures below 600° C, chloritoid was not produced in runs lasting up to four months,

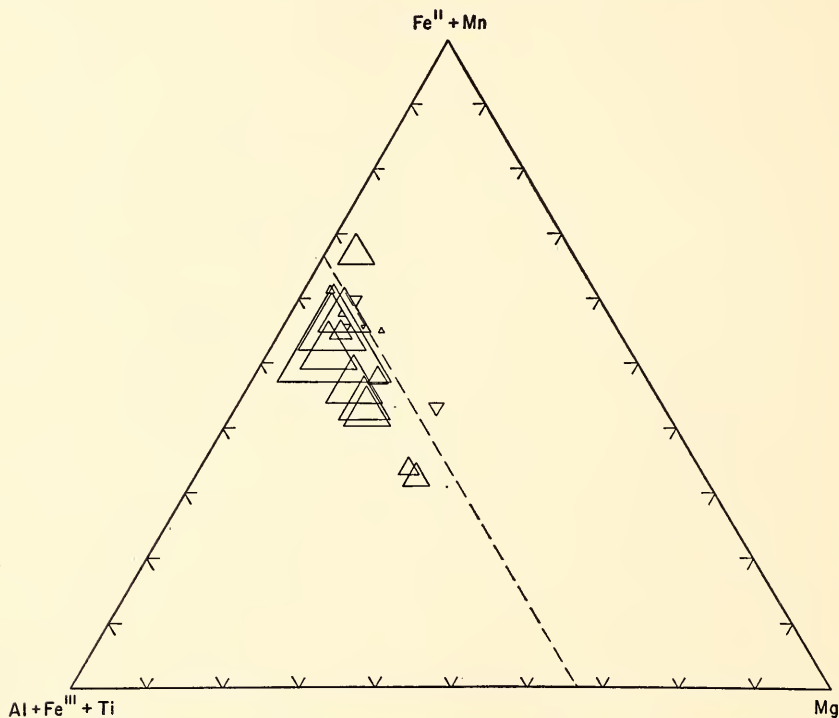


FIG. 31. Chemical composition of natural chloritoid based on atomic variations in the mixed octahedral layer,  $[(\text{Fe}^{II}, \text{Mg}, \text{Mn})_2(\text{Al}, \text{Fe}^{III})]$ , of the structure.

the same as that found by Schairer and Yagi for the anhydrous chloritoid composition in the system  $\text{FeO}-\text{Al}_2\text{O}_3-\text{SiO}_2$  (Geophysical Laboratory Paper No. 1193). In most of the runs above 600° C, however, the metastable assemblage hercynite + quartz is produced, iron cordierite crystallizing only in long runs. The same results have been obtained with many mixtures of the chloritoid composition. This difference in breakdown products between 10,000 bars and 2000 bars leads one to suspect that

starting with a large number of mixtures of the requisite composition. The crystalline products formed were quartz + hercynite-cordierite solid solutions + a phase on the greenalite ( $3\text{FeO} \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$ )—pseudothuringite ( $2\text{FeO} \cdot \text{Al}_2\text{O}_3 \cdot \text{SiO}_2 \cdot 2\text{H}_2\text{O}$ ) join (see fig. 30), probably having a composition close to daphnite ( $7\text{FeO} \cdot 2\text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2 \cdot 6\text{H}_2\text{O}$ ) or to chamosite (similar in composition to daphnite but having some of the iron oxidized). The X-ray pattern of this phase, which grows

very quickly, indicates that it has a structure similar to that of either chlorite or kaolinite, but which one has not been distinguished with certainty. Brindley has found that material called chamosite has two structures, one similar to chlorite and the other to kaolinite. This phase of doubtful structure may be an iron analogue of aluminous serpentine, the low-temperature kaolinite-like form of clinocllore composition made by Yoder in the system  $\text{MgO}-\text{Al}_2\text{O}_3-\text{SiO}_2-\text{H}_2\text{O}$  (Geophysical Laboratory Paper No. 1199).

The stability field of chloritoid is defined not only by the temperature, the total pressure, and the  $\text{H}_2\text{O}$  pressure, but also by

the partial pressure of oxygen (see section on Stability of Hydrous Iron Silicates). Hence, it may well be that the chlorite- or kaolinite-like phase is stable at higher partial pressures of oxygen than chloritoid, but within the same temperatures and total pressures. This statement is based on the observation of Dschang that the iron in chlorites can be oxidized without destroying the structure.

These preliminary experiments show that chloritoid can form under hydrothermal conditions in the laboratory without the aid of stress. They also show that its stability range is not as restricted as was formerly thought.

## CRYSTALLOGRAPHY

G. DONNAY

### HIGH-ORDER TRANSITIONS IN MINERALOGY

Polymorphic transformations, structural changes that take place without modification of chemical composition, have long been known in mineralogy; they accompany changes in temperature, pressure, and magnetic field. Both first-order and high-order transitions are represented among them: for example, quartz to tridymite and  $\alpha$ -quartz to  $\beta$ -quartz, respectively. Other high-order polymorphic transitions found when the temperature is raised are a result of the concomitant disordering, of the setting-in of rotation of ionic groups, or of the lining-up of magnetic dipoles that marks the passage from unmagnetized to magnetized states in ferromagnetic substances. In such cases the crystal structures of the various forms have been determined and are known to be different.

It seems to have been largely taken for granted, however, that the crystal structure of a mineral with a range of composition remains essentially the same throughout this range. Slight changes in the atomic co-ordinates would naturally result from substitutions, additions, or omissions, but these changes would be continuous functions of the composition up to the limit of solid solubility, where a second phase ap-

pears. Consequently it has been the accepted practice to determine the crystal structure of a mineral at only one (known) composition. The evidence presented below shows that this procedure is unsafe, for the underlying assumption is not always justified.

According to Ehrenfest's definition, a high-order transition corresponds to a discontinuity in any one of the derivatives of the Gibbs free energy  $G$ , the first-order transition corresponding to a discontinuity in  $G$  itself. From the definition of  $G$ ,  $G=E-TS$ , it follows that  $dG=VdP-SdT$  and that  $(\partial G/\partial P)_T=V$ . But  $G$  may also be considered a function of  $T$ ,  $P$ , and composition  $x$ , so that we may write the second derivative  $(\partial^2 G/\partial P \partial x)_T=(\partial V/\partial x)_T$ . Thus a singularity in the curve of  $V$  against  $x$ , for example, indicates a second-order transition. (The convention is to set the order of the transition equal to the order of the lowest derivative of  $G$  that shows a discontinuity.)

A plot of lattice parameter against composition for synthetic nepheline ( $\text{K}_{x-1}\text{Na}_{1-x}\text{Al}_8\text{Si}_8\text{O}_{32}$ ), previously reported by Smith (Year Book No. 52, 1952-1953, p. 55), shows two singularities. The function that is of interest from the thermodynamic

point of view is the volume, as shown by the above equations. The curve of cell volume against composition also shows two singularities at  $x = \frac{1}{4}$  and  $x = 2$ . These are the compositions at which second-order transitions take place. They divide the compositional range into three segments, corresponding to three structural variations for which the term "subphases" might be found useful.

The crystal on which the crystal structure of nepheline was determined (Buerger, Klein, and Donnay, 1954) and refined (Hahn and Buerger, 1955) came from the Monte Somma locality and had a potassium content of  $2.0 \pm 0.2$  ions per cell ( $x = 2$ ). The crystal structure itself exhibits a feature indicating that the composition is near the transition point. It consists in a statistical displacement of two of the framework oxygens from a twofold position *on* the threefold axes to a sixfold position *near* the threefold axes. On the electron-density projection each of the six peaks has roughly the height of one oxygen peak. It will be necessary to determine the crystal structures of two crystals, with compositions on opposite sides of the transition composition, in order to evaluate the structural changes involved. For example, a high-potassium nepheline ( $x > 2$ ) should be compared with a low-potassium nepheline ( $x < 2$ ). Structure-sensitive physical and chemical properties may be expected to show sudden changes at the transition compositions. The chemical reactivity, in particular, should increase sharply, according to Hedvall's rule.

It will be recalled that the X-ray study of high-temperature alkali feldspars revealed a change in symmetry from monoclinic to triclinic. The absence of a two-phase region had already led to the conclusion that this symmetry change corresponds to a high-order transition (Year Book No. 51, 1951-1952, and Geophysical Laboratory Paper No. 1179). The curve of cell volume against composition, however, did not show any break, so that in

this case the order of the transition must be greater than 2.

Other minerals that show extensive ranges of solid solution can be expected to show high-order transitions. For tourmaline, for example, the two somewhat different crystal structures that have been reported in the literature (Donnay and Buerger, 1950; Ito and Sadanaga, 1951) may well be those of distinct "subphases."

#### DIGENITE

Digenite has been considered a cubic mineral ever since it was first described in 1844. As was reported last year, X-ray diffraction patterns taken by single-crystal techniques indicated that the side of the cubic cell had to be multiplied by 5 (its volume by 125) to account for the new observations. In pursuing that work, much effort was spent in trying to find a crystal structure for digenite that would be based on the large cubic cell. But it proved impossible to account for the many structural absences observed among the X-ray reflections. The key to the interpretation of this unusual phenomenon has been found in the similar case of sodium superoxide III, described by Carter and Templeton (1953). Twinning is responsible. Re-examination of the films reveals slight intensity differences between corresponding reflections from different individuals of the twin. The striking cubic symmetry thus turns out to be "twin symmetry," not "crystal symmetry." The "octahedron" consists of four or eight rhombohedral crystals, with  $a = 3.92$ ,  $c = 48.0$  Å,  $c/a = 12.25$ ,  $Z = 3$ , diffraction aspect  $R^{**}$ . Each crystal has its  $c$  axis directed along a body diagonal and its  $a$  axes parallel to the face diagonals of the simulated cubic cell. Available atomic positions and intensity considerations leave  $R_{32}$ ,  $R_{\bar{3}}$ , and  $R_3$  as the only possible space groups. In order to attack the crystal structure of the new digenite, X-ray intensity data would have to be obtained from an untwinned crystal. Kullerud, who was the first to succeed in growing the octahedral twins, finds it even more



difficult to produce truly single crystals. It appears that the twin is definitely more stable than the crystal. This situation may be correlated with the astonishing morphology of the edifice, in which the faces obey the Law of Simple Indices when referred to the cubic cell of the twin lattice, but must be assigned complicated symbols when referred to the true cell of the crystal lattice. A search through the literature discloses the fact that only one compound possesses a rhombohedral cell as steep as that of digenite, namely ZnS type 15R, described by Frondel and Palache (1950). It is a perplexing observation that, although the two compounds have the same number of sulfur atoms per cell, digenite contains 27 copper atoms, whereas ZnS type 15R contains 15 zinc atoms per cell. No great structural analogy between the two compounds can thus be hoped for.

#### KLOCKMANNITE

The X-ray reflections observed on the klockmannite films, collected on Kullerud's synthetic crystals (Year Book No. 54), showed structural absences of great regularity, reminiscent of the digenite situation. In the plane of the (*HK*0) reflections, for instance, *H* and *K* are multiples of 13 for the strong reflections, so that only 1 out of every 169 possible reflections appears. Each strong reflection, however, is surrounded by 12 weak reflections outlining a dodecagon, and no other weak reflections are observed. By his work on optical syntheses, H. Lipson has shown that similar patterns can be the result of domain structures, the various domains being displaced one with respect to another. In view of the striking resemblances between the klockmannite pattern and those he himself had studied, Lipson has kindly agreed to cooperate in the present investigation. He will analyze the klockmannite data on his optical setup in Manchester.

#### SOLID SOLUTION

Omission solid solution, which is frequent in crystal structures of the semi-

metallic type, is rare in silicates. In the system  $\text{NaAlSiO}_4\text{—NaAlSi}_3\text{O}_8$ , sodium sites are known to be vacant in the nepheline structure. A single crystal (prepared by MacKenzie) having the maximum amount of omission solid solution in the above system shows no change in cell dimensions from those of pure  $\text{NaAlSiO}_4$ . As in digenite, where the sulfide framework was not affected by the removal of copper, the silicon-aluminum framework is not altered by the removal of sodium. It follows that, in contradistinction to most substitution solid solutions, the above omission solid solutions do not lend themselves to a determination of chemical composition from variations in cell dimensions.

Substitution and omission combine in the system  $\text{NaAlSiO}_4\text{—CaAl}_2\text{Si}_2\text{O}_8$  so that two sodium ions are replaced by one calcium ion and one vacancy. Crystalline powders showing the maximum amount of this combined solid solution, prepared by Schairer, were used in this work. In spite of the substitution of calcium for sodium, the cell was found to remain the same in size and shape.

Double substitution, calcium for sodium and aluminum for silicon, has long been known in plagioclases; it has also been reported in the system  $\text{NaAlSiO}_4\text{—CaAl}_2\text{O}_4$  by J. R. Goldsmith (1949). The latter kindly provided a number of specimens on which cell dimensions were determined and found to vary slightly and linearly with composition. The cell expands from 720 to 729 Å<sup>3</sup> when 57.5 atomic per cent of sodium and silicon are replaced by calcium and aluminum respectively.

#### BIOCRISTALLOGRAPHY

The term biocrystallography was not much used until the Third International Congress of Crystallography (Paris, 1954), where Dr. von Phillipsborn presented a very challenging outline of the many fields of biological and paleontological endeavor on which X-ray diffraction techniques might be expected to throw light. An excursion has been made into this hitherto

unfamiliar territory, with rewarding findings. The work was carried out jointly with Dr. A. R. Moore, of the Hopkins Marine Station, Pacific Grove, California. Its purpose was to contribute to our knowledge of echinoderm tests.

The nature of the skeletons of echinoderms is of special interest to biologists, because it has been suggested that Echinodermata may have been invertebrate ancestors of the vertebrates. The possibility has been mentioned that calcium hydroxy apatites, commonly present in vertebrate skeletons, may make their appearance in the skeletons of echinoderms. If this were proved it would be a strong argument on the point at issue.

Tiny skeletons of plutei of *Strongylocentrotus purpuratus*, 0.1 mm long, were examined on the precession camera. They are single crystals of calcite, with cell dimensions that suggest no appreciable replacement of calcium by magnesium. The length of the skeleton coincides with the *c* axis of the crystal; a lateral rod extending out at an angle to the main rod is part of the same crystal, so that the *c* axis cannot be parallel to the length of the lateral rod. Plutei reared in trypsin in sea water show a doubling of the skeletal rod; again, the whole skeleton was found to be constituted by a single crystal. In order to ascertain the control, if any, that foreign ions in the solution might exert on which polymorphic form of calcium carbonate is used by the organism to build its skeleton, Moore grew plutei in a dilute solution of strontium chloride in sea water. Strontium is known to substitute for calcium in minerals and

to stabilize aragonite over calcite. The plutei, however, were not affected; they remained single crystals of calcite.

Adult skeletons consist of hexagonal plates. These were also studied on the precession camera. Each plate is a single crystal of calcite, but the surface of the plate is not parallel to any crystal face, nor does its hexagonal outline follow crystallographic directions. Moreover, the orientation of the crystal was found to change drastically and unpredictably from one plate to the next. The lattice orientation changes slightly across a plate and appears to follow its curvature. The presence of such a curved crystal may explain why it is impossible to cleave a plate despite the fact that normal calcite is very easily cleavable. Determination of cell dimensions leads to an estimated 13 atomic per cent of magnesium, considerably more than the mineral can take into solid solution when it is not part of a living organism.

Similar X-ray studies were carried out on the bony parts of an Aristotle's lantern of *Strongylocentrotus franciscanus*. These parts consist of bundles of calcite fibers, each of which is a curved single crystal. Their magnesium content, about 6 atomic per cent, is less than that of the plates, but is appreciable.

As to calcium hydroxy apatite, none could be detected in any of the above structures.

It may be concluded that there are problems in which crystallographic techniques, especially X-ray diffraction, can be of real help to biologists and paleontologists.

## TRANSPORT OF SOLIDS BY STEAM AT HIGH PRESSURE

G. W. MOREY

The solution of solids by gases is a general phenomenon, and a survey of the literature brings to light some interesting examples. Ice is soluble in air! At  $-35^{\circ}$  C and 200 atm pressure, the compressed air contains 3 times as much water as would be expected from the vapor pressure of ice.

Solid carbon dioxide, "dry ice," is soluble in compressed air! At  $-150^{\circ}$  C and 200 atm pressure, the compressed air contains 1481 times as much carbon dioxide as would be calculated from its vapor pressure. With sodium chloride and steam at  $500^{\circ}$  C, 5000 psi pressure, at this Labora-

tory we have obtained gases which contained 10 per cent of dissolved NaCl. This is 100 million times the amount corresponding to its vapor pressure. Even with such a nonvolatile material as aluminum oxide we have found solubilities of the order of 10 parts per million. Uranium oxide has the lowest solubility in superheated steam of any material we have tried,  $\frac{1}{2}$  part per million at 500° C and 30,000 psi.

New work with quartz at 600° C and 45,000 psi steam pressure has demonstrated a solubility of 1.2 per cent, a value which agrees well with the extrapolation of previous results at lower pressures.

Last year we reported a study of the action of hot water on some feldspars, and this year the technique has been extended to the study of the carbonates: calcite,  $\text{CaCO}_3$ ; magnesite,  $\text{MgCO}_3$ ; and dolomite,  $\text{CaMg}(\text{CO}_3)_2$ . In these experiments, pure water, freed from carbon dioxide by passage over a suitable ion-exchange resin, is passed over the carbonate contained in a stainless-steel tube heated in a furnace. The pressure is held at about 3000 psi, above the vapor pressure of water at the highest temperature used in these experiments, thus ensuring that there was no steam, only

liquid water. The solubility of calcite at 25° C is about 20 parts per million; it changes little up to about 150°, where it begins to fall uniformly to about 7 parts per million at 350°. The solubility of magnesite increases with temperatures up to about 150°, but above that temperature a new phenomenon occurs. X-ray studies of the materials showed that below 150° the magnesite was not altered, but above 150° it was decomposed, with formation of brucite,  $\text{Mg}(\text{OH})_2$ , and liberation of carbon dioxide. Dolomite also is decomposed by water above 200°, but the exact temperature at which decomposition begins under the conditions of our experiments has not been determined. At and below 209°, X-rays show the dolomite pattern; above this temperature, brucite and calcite. Analysis shows, however, that at 179° the solution contains some free carbon dioxide, and it is possible that the decomposition had begun at that temperature, but that below 209° the rate was too small for the brucite to show in the X-ray pattern. It is planned to make some long runs to test this point. An extension of this work, using water containing a small percentage of carbon dioxide, is under way.

## ANHYDROUS SILICATE SYSTEMS

### MELTING RELATIONS OF THE COMMON ROCK-FORMING OXIDES

J. F. SCHAIRER

Laboratory studies of the mutual melting behavior of the common rock-forming oxides  $\text{SiO}_2$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{FeO}$ ,  $\text{Fe}_2\text{O}_3$ ,  $\text{CaO}$ ,  $\text{MgO}$ ,  $\text{Na}_2\text{O}$ , and  $\text{K}_2\text{O}$  have been an important adjunct to the observations of the field geologist and petrographer. During the past fifty years systematic studies of the melting, crystallization, and stability relations in the principal systems of rock-forming oxides have been conducted at the Geophysical Laboratory. These phase-equilibrium studies have yielded much information on the compositions and mutual melting and crystallization relations of the rock-

forming olivines, pyroxenes, pyroxenoids, melilites, feldspars, and feldspathoids. In addition, these data have proved widely useful in technology, particularly in the fields of refractories, porcelains, glass manufacture, Portland cement, and slag metallurgy, and even have provided basic information for such diverse items as welding fluxes, boiler-scale deposits, and the fusibility of coal-ash slags.

During the past year, in a paper entitled "Melting Relations of the Common Rock-Forming Oxides," the progress in this field, based principally on research in silicate systems at the Geophysical Laboratory, has been summarized and reviewed, by invitation, in the Orton Memorial Lecture delivered at the Annual Meeting of the



American Ceramic Society in New York in April 1956. The manuscript has been submitted for publication.

# THE SYSTEM $\text{Na}_2\text{O}-\text{MgO}-\text{SiO}_2$

J. F. SCHAIRER, H. S. YODER, JR., AND  
A. G. KEENE

The systems  $\text{Na}_2\text{O}-\text{FeO}-\text{SiO}_2$  and  $\text{Na}_2\text{O}-\text{MgO}-\text{SiO}_2$  were the only ternary systems of common rock-forming oxides for which reliable phase-equilibrium data were not available. A knowledge of the phase relations in these anhydrous systems was prerequisite to studies of these same systems with water as an additional component. A study of the hydrous systems should elucidate the complex compositions of and relations between the alkali pyroxenes and amphiboles which are major constituents of certain alkaline rocks and minor mineral constituents of many other igneous rocks.

Four years ago a reconnaissance of the system  $\text{Na}_2\text{O}-\text{FeO}-\text{SiO}_2$  was begun. This system proved to be very complex and difficult experimentally. Many new crystalline ternary compounds were encountered whose optical properties and X-ray patterns were obtained, but whose compositions could not be ascertained because of the complexity of the relations and because of the change in composition of melts by reaction with iron crucibles used as containers. At this stage it became obvious that further progress in the system  $\text{Na}_2\text{O}-\text{FeO}-\text{SiO}_2$  required a knowledge of the comparable system  $\text{Na}_2\text{O}-\text{MgO}-\text{SiO}_2$ . This last system had been studied by Russian investigators, but reconnaissance experiments in our laboratory showed that the data were inadequate and unreliable and that a new study of the system was necessary.

Our studies of the system  $\text{Na}_2\text{O}-\text{MgO}-\text{SiO}_2$  are now completed, and a manuscript is nearly ready for publication. Preliminary diagrams for this system were given in two previous annual reports (Year Book No. 52, 1952-1953, pp. 62-64; No. 53, 1953-1954, pp. 123-125).

The phase diagram shows nine ternary compounds in addition to the binary compounds from the limiting binary systems. Seven of these ternary compounds have incongruent melting points but show no polymorphism or solid solutions. Two ternary compounds,  $2\text{Na}_2\text{O} \cdot 2\text{MgO} \cdot 3\text{SiO}_2$  and  $\text{Na}_2\text{O} \cdot \text{MgO} \cdot \text{SiO}_2$ , have congruent melting points and show polymorphism. There is a complete series of solid solutions between these two compounds. An examination of the complex relations in the ternary system  $\text{Na}_2\text{O}-\text{MgO}-\text{SiO}_2$  shows why a careful and detailed study was necessary before we could proceed with our study of the system  $\text{Na}_2\text{O}-\text{FeO}-\text{SiO}_2$  or proceed with the inclusion of water as an additional component.

## PHASE-EQUILIBRIUM RELATIONS IN THE QUADRILATERAL $\text{MgSiO}_3-\text{CaMgSi}_2\text{O}_6$ $-\text{CaFeSi}_2\text{O}_6-\text{FeSiO}_3$

J. F. SCHAIRER, F. R. BOYD, JR., AND  
H. P. EUGSTER

The pyroxenes and pyroxenoids present exceptional complexity both of crystalline modifications and of chemical composition. They are a series of polycomponent solid solutions, some of the components of which are completely miscible and others only partly so. The important components of many natural pyroxenes are given in table 13.

The three most important components

TABLE 13

### COMPONENTS OF THE ROCK-FORMING PYROXENES

$\text{CaSiO}_3$ .....	Wollastonite, pseudowollastonite
$\text{MgSiO}_3$ .....	Enstatite, clinoenstatite, protoenstatite
$\text{CaSiO}_3 \cdot \text{MgSiO}_3$ ....	Diopside
$\text{FeSiO}_3$ .....	Ferrosilite, clinoferrosilite
$\text{CaSiO}_3 \cdot \text{FeSiO}_3$ ....	Hedenbergite
$\text{MnSiO}_3$ .....	Rhodonite, bustamite
$\text{CaSiO}_3 \cdot \text{MnSiO}_3$ ....	Johannsenite
Molecules containing	
$\text{Al}_2\text{O}_3$ .....	Augites
$\text{Fe}_2\text{O}_3$ .....	Augites and babingtonite
$\text{TiO}_2$ .....	Titaniferous augites
$\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2$ ..	Jadeite
$\text{Na}_2\text{O} \cdot \text{Fe}_2\text{O}_3 \cdot 4\text{SiO}_2$ ..	Acmite, aegirite



are the metasilicates  $\text{CaSiO}_3$ ,  $\text{MgSiO}_3$ , and  $\text{FeSiO}_3$ . The composition triangle,  $\text{CaSiO}_3$ — $\text{MgSiO}_3$ — $\text{FeSiO}_3$ , which is given as figure 32, shows the chemical relations between diopside, hedenbergite, and the simple metasilicates. Some years ago at the Geophysical Laboratory, Bowen, Schairer,

and the lime-iron olivines. In a like manner, in a study of the system  $\text{MgO}$ — $\text{FeO}$ — $\text{SiO}_2$ , Bowen and Schairer (1935) presented complete data on the chemical compositions, optical properties, and mutual melting and stability relations of the  $\text{MgSiO}_3$ — $\text{FeSiO}_3$  pyroxenes, including both the clino-

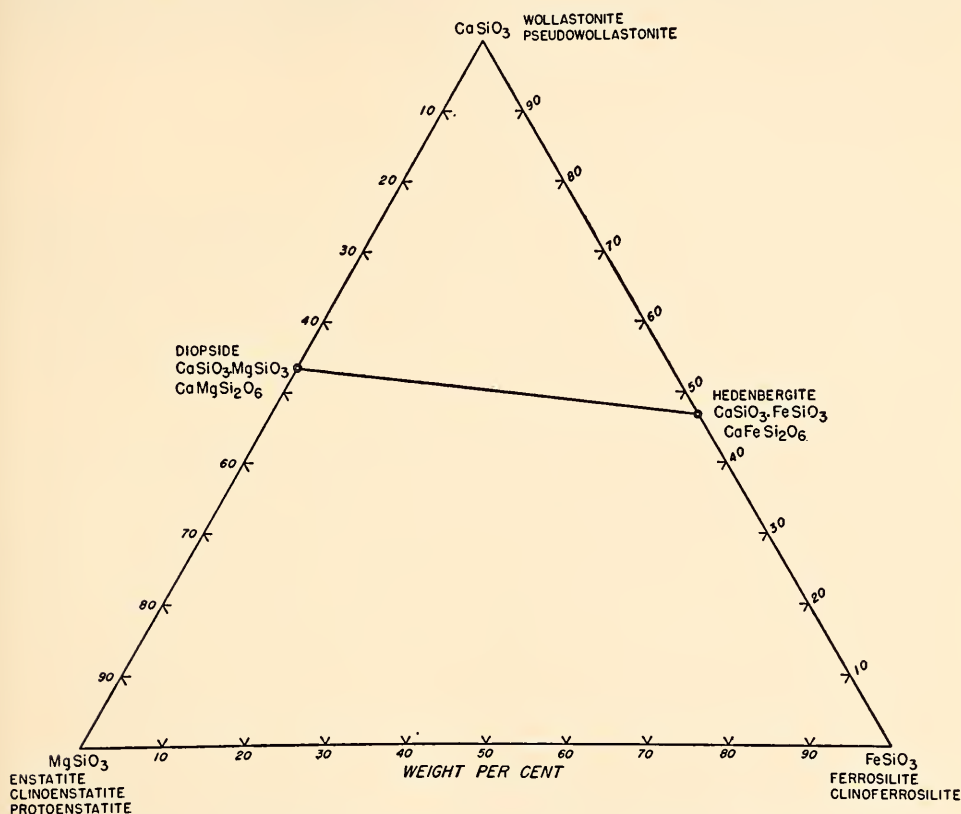


FIG. 32. Equilateral triangle  $\text{CaSiO}_3$ — $\text{MgSiO}_3$ — $\text{FeSiO}_3$  showing the composition relations between diopside, hedenbergite, and the simple metasilicates.

and Posnjak (1933) studied the system  $\text{CaO}$ — $\text{FeO}$ — $\text{SiO}_2$  and described the chemical compositions, optical properties, and mutual melting and stability relations of the lime-iron pyroxenes and pyroxenoids (all compositions between  $\text{CaSiO}_3$  and  $\text{FeSiO}_3$ ), including pseudowollastonite ( $\alpha$ - $\text{CaSiO}_3$ ), wollastonite ( $\beta$ - $\text{CaSiO}_3$  solid solutions with up to 76 per cent  $\text{FeSiO}_3$ ), hedenbergite (with hedenbergite solid solutions containing up to 80 per cent  $\text{FeSiO}_3$ ),

pyroxenes and the orthopyroxenes, and their relations to the magnesia-iron olivines. Schairer and Bowen (1942) showed that in the system  $\text{CaSiO}_3$ — $\text{CaMgSi}_2\text{O}_6$  there is little or no solid solution of diopside in pseudowollastonite ( $\alpha$ - $\text{CaSiO}_3$ ) or of  $\text{CaSiO}_3$  in diopside, but that wollastonite ( $\beta$ - $\text{CaSiO}_3$ ) may take up to a maximum of 22 weight per cent of diopside in solid solution. Bowen (1914), in his study of the system diopside—ferrosilite—silica,

showed the presence of a complete series of solid solutions between diopside and clinoenstatite at elevated temperatures and gave their optical properties and complete melting relations. He did not, however, study the unmixing of these solid solutions at lower temperatures. This unmixing was studied by Leon Atlas (1952) at the University of Chicago.

The results of Atlas are now being checked by long quenching runs and care-

ful X-ray determinations on a series of compositions between  $\text{MgSiO}_3$  and  $\text{CaMgSi}_2\text{O}_6$ . A series of melts are now being prepared in the quadrilateral  $\text{MgSiO}_3$ — $\text{CaMgSi}_2\text{O}_6$ — $\text{CaFeSi}_2\text{O}_6$ — $\text{FeSiO}_3$ , for phase-equilibrium studies during the next year. A study of the joins  $\text{MgSiO}_3$ — $\text{FeSiO}_3$  and  $\text{CaSiO}_3$ — $\text{FeSiO}_3$  at temperatures below those studied by previous investigators is in progress.

## STATISTICAL PETROLOGY

F. CHAYES

### GRANITE AND PETROGENY'S RESIDUA SYSTEM

In any natural science there ought to be constant and stimulating debate and cooperation between naturalists and experimentalists. Such a relation demands a common language and most particularly a common nomenclature. Agreement and disagreement are equally irrelevant when we are not sure we are talking about the same thing. This problem has never been especially critical in petrology; until recently, experimentalists were so few and far between that they had to make do with whatever nomenclature the descriptive petrographer or field geologist provided. The situation is changing rapidly, however; the amount and quality of laboratory work increase steadily, and in certain areas of petrography the profit to be derived from a standardized and widely agreed upon nomenclature would greatly outweigh the temporary irritation almost certain to be encountered in achieving it. This need is nowhere more apparent than in the study of granites, by all odds, and however defined, the most abundant intrusive rocks.

The basis of classification of any natural rock type must always be primarily geological and petrographic. In any natural classification and naming of granites, however, careful attention must be given to what is now known of phase relations and liquidus surfaces in the silica-rich part of the system  $\text{NaAlSi}_3\text{O}_8$ — $\text{KAlSi}_3\text{O}_8$ — $\text{SiO}_2$ — $\text{H}_2\text{O}$ . No new classification or reorganization of existing nomenclature that ignores

this information will be of much assistance in promoting the kind of understanding and interaction between field and laboratory workers that is taken so much as a matter of course in numerous fields of inquiry and is so conspicuous by its absence from ours.

Although only fragmentary data were available for the silica-rich portion of the "dry" system  $\text{NaAlSi}_3\text{O}_8$ — $\text{KAlSi}_3\text{O}_8$ — $\text{SiO}_2$  at the time of Bowen's original suggestion, the notion of a thermal trough essentially paralleling the  $\text{NaAlSi}_3\text{O}_8$ — $\text{SiO}_2$  margin and centered along a line connecting the minima is confirmed both by Schairer's most recently published diagram for the system (fig. 33) and by some of his as yet unpublished data. The thermal valley delineated by Bowen is a narrow band extending from the ternary minimum at the base of the steep fusion slope of nepheline to the ternary minimum at the base of the even steeper fusion slope of silica. It slopes gently toward each of these minima from a maximum at the binary minimum on the feldspar join. Except for a narrow zone immediately under the boundary of the silica field, the potassic margin of the valley is determined by the steep fusion slope of leucite. Relations on the sodic side are persuasive if less compelling. Bowen's valley is thus a region of low thermal relief and temperature bounded at both ends and along most of both sides by regions of considerably higher relief and temperature.

The situation changes drastically with

increasing  $\text{H}_2\text{O}$  pressure. At 1000 kg/cm<sup>2</sup>, for instance, the first pressure for which sufficient data have been published (see fig. 34, taken from the report of Bowen and Tuttle in Year Book No. 51, p. 39), the principal topographic feature revealed by the isotherms is a broad asymmetric valley essentially paralleling the

portance even in such a mundane matter as the use of the word "granite." For nearly a hundred and fifty years those who have paid any attention at all to modal composition have restricted the name to highly feldspathic rocks rich in quartz. Originally, the feldspar was supposed to be orthoclase, and though the restriction actu-

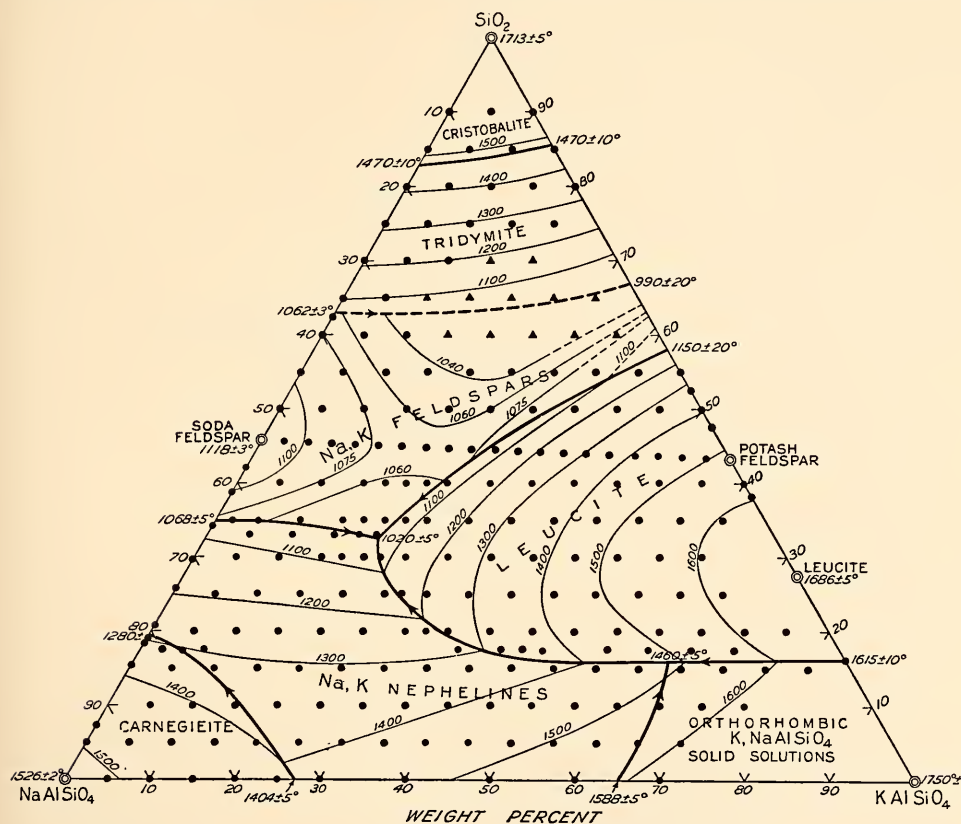


FIG. 33. Liquidus surface in the system  $\text{NaAlSiO}_4\text{--KAlSiO}_4\text{--SiO}_2$  (Schairer)

margin of the quartz field; what was formerly the major valley connecting the binary and ternary minima in the anhydrous system is now only a minor indentation. The region immediately beneath the margin of the quartz field, extending from one side of the diagram to the other, is characterized throughout by low temperatures.

The shape and position of this thermal valley are of considerable petrographic im-

portance even in such a mundane matter as the use of the word "granite." (It is not uncommon, for instance, in dictionary definitions.) That granitic rocks devoid of plagioclase are extremely scarce has been known for a long time, but the tendency to restrict the name granite to rocks in which potassium feldspar at least *exceeds* plagioclase is still widespread. A whole suite of names has been developed to denote rocks which dif-

fer from granite only in the extent of the relative dominance of plagioclase over alkali feldspar. For the most part the amounts of the two feldspars are either inadequately determined or inferred from occasional chemical analyses. The really important point is whether these names are to be regarded as denoting variants of

ley, essentially paralleling the margin of the quartz field, is evident. The line in figure 35B shows the trace of the thermal valley as originally proposed by Bowen for the "dry" system.

For the points,  $X$  = volume per cent of quartz,  $Y$  = volume per cent of plagioclase,  $Z$  = volume per cent of alkali feldspar,

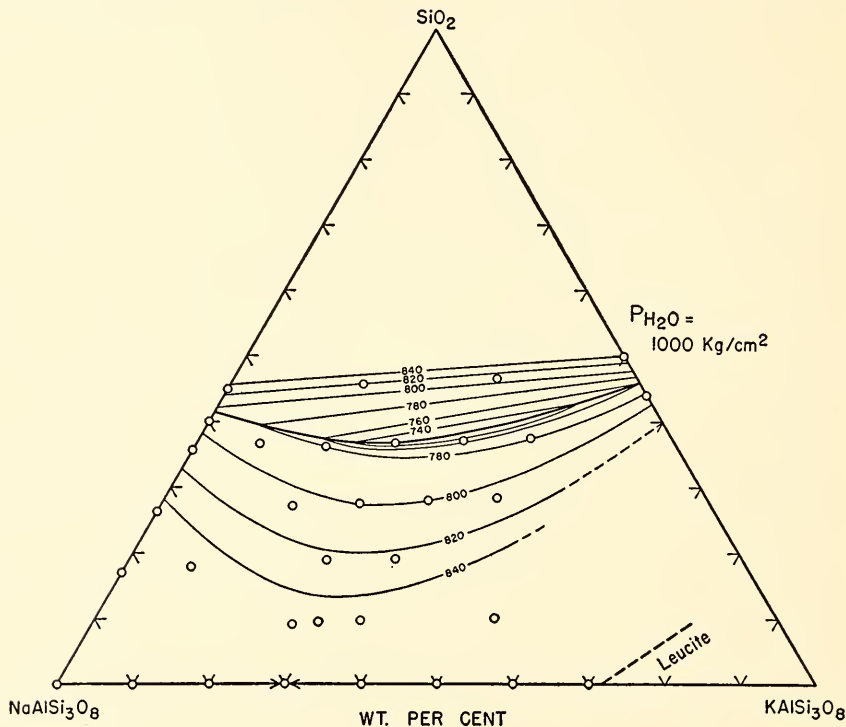


FIG. 34. Projection of liquidus surface in the system  $\text{SiO}_2$ — $\text{NaAlSi}_3\text{O}_8$ — $\text{KAlSi}_3\text{O}_8$ — $\text{H}_2\text{O}$  at  $P_{\text{H}_2\text{O}} = 1000 \text{ kg/cm}^2$  (Bowen and Tuttle).

granite or rocks fundamentally different from granite.

A partial answer to this question is provided in figure 35, which requires considerable explanation. In this figure the coordinates differ for points and lines. For the lines,  $X$ ,  $Y$ , and  $Z$  are, respectively, weight per cent of  $\text{SiO}_2$ ,  $\text{NaAlSi}_3\text{O}_8$ , and  $\text{KAlSi}_3\text{O}_8$ . The heavy line in figure 35A is the margin of the quartz field at  $1000 \text{ kg/cm}^2$ , and the light lines are isotherms on the liquidus at that pressure, as shown in figure 34. The orientation of the main val-

ley after elimination of other constituents; e.g.,  $X = \text{quartz}/(\text{quartz} + \text{feldspar})$ , etc. Each point is an average value of the ternary ratio for some specific granite mass; the number of specimens on which each average is based is given approximately by the symbol, as described in the legend. There are 42 points in all, based on a total of 414 specimens collected in the course of the last nine years. In most of these rocks, color index is very low, and, except for the possibility of occasional excessive calcium in plagioclase, discussed below, they must



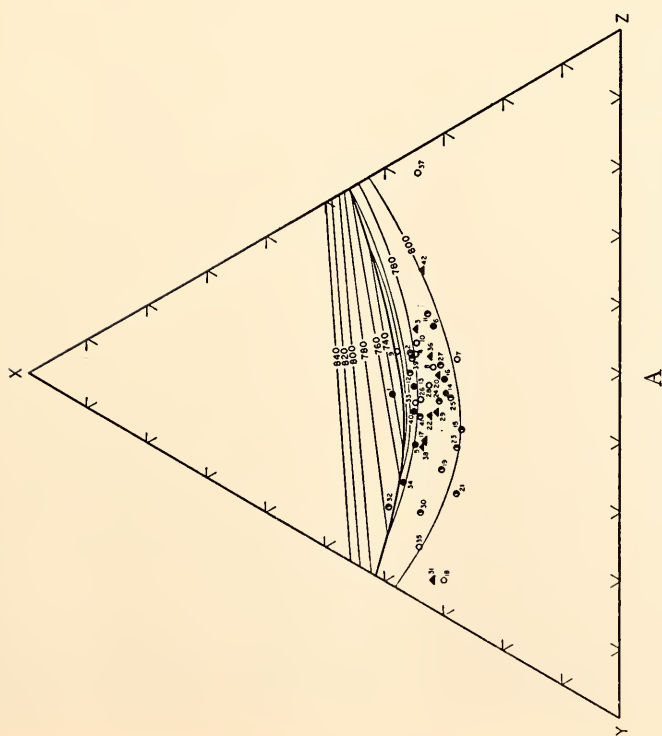
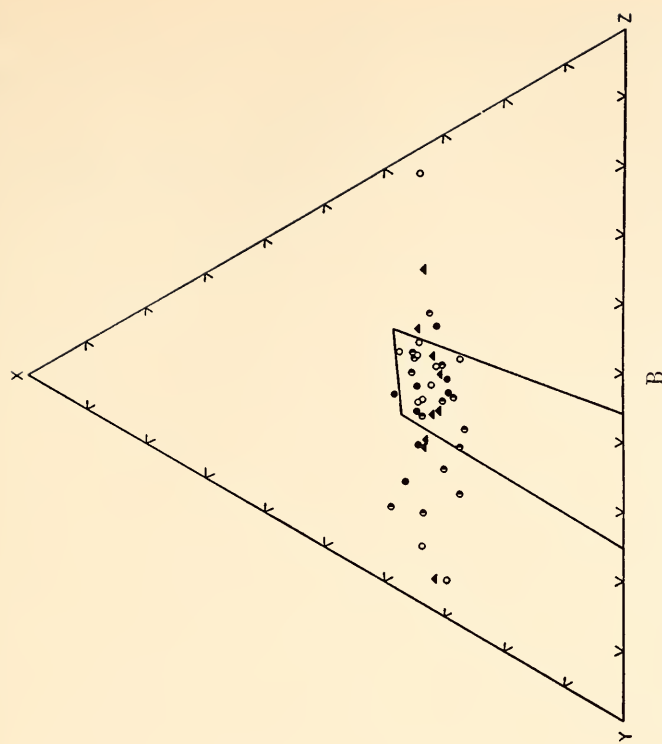


FIG. 35. Average modal composition of some granite masses. A, contours from figure 34; B, trace of Bowen's thermal valley in anhydrous system. Co-ordinates. Lines: X,  $\text{SiO}_2$ ; Y,  $\text{NaAlSi}_3\text{O}_8$ ; Z,  $\text{KAlSi}_3\text{O}_8$ ; all in weight per cent. Points: X = quartz/(quartz + feldspar); Y = plagioclase/(quartz + feldspar); Z = alkali feldspar/(quartz + feldspar); all in volume per cent. Symbols. Open circle,  $n < 5$ ; half-solid circle,  $5 \leq n < 10$ ; solid circle,  $10 \leq n < 15$ ; solid triangle,  $n \geq 15$ ; where  $n$  is the number of specimens on which the average is based. The total number of specimens is 414.

Locality key. Nos. 1-19, New England; 20-25, southeastern United States; 26-29, Texas; 30-34, California; 35-36, Minnesota, South Dakota; 37, Wisconsin; 38, Lanhelein; 39, Carmanellis; 40, Sierra de Guadarrama; 41, Randolph, Massachusetts; 42, Arran.



easily satisfy the requirements set up by Bowen and Tuttle in similar plotting of chemically analyzed materials (see, for instance, Year Book No. 51, p. 41).

The use of a common diagram for reduced modal analyses and phase-equilibrium isotherms involves a number of assumptions, of which at least three must be mentioned here. The modes have not been transformed from volume to weight per cent; with rare exceptions the adjusted weights would not differ significantly from direct volumetric proportions. No allowance has been made for the microscopic or submicroscopic albite in alkali feldspar; it is assumed that this has exsolved from an initially homogeneous K-Na feldspar. Except in the points at the extreme right (nos. 37 and 42) the amount is small, probably of the order of 10 per cent (see Year Book No. 53, p. 136, for detailed information on the New England granites). No adjustment has been made for the Ca content of plagioclase. Except for a few (not all) of the points at the left, this is also small, though appreciably larger than the exsolved albite of alkali feldspar. The plagioclase of most of these rocks generally contains 10 to 20 per cent An; except where plagioclase greatly exceeds alkali feldspar, extraction of anorthite before calculation of the ternary ratio will not shift the position of the ratio by a significant amount. The shift will always be toward XZ, but it will rarely be more than a few per cent.

It is evident from the diagram that an overwhelming majority of the points fall in the thermal valley on the feldspar side of the field boundary, where they would be expected to lie if granite magmas were generated by crystal fractionation or partial melting. A very wide range of Y/Z exists, as would be anticipated from the shape of the region of low temperature in the thermal diagram for the hydrous crystallization; the range is much broader than would be expected either from the original valley delineated in the anhydrous system or from too strong an emphasis on ap-

proach to some *specific* minimum melting composition.

All the points shown in figure 35 describe rocks of granitic appearance, texture, and geology. Some have been called quartz monzonites, some granodiorites, some adamellites, some quartz diorites, and at least one is described as tonalite. All have also either been called granite or repeatedly referred to as granitic. The strict petrographic nomenclature is so confused, however, that according to one prominent classification only 14, and according to another only 2, *should* be called granite. To everyone but the petrographic systematist these rocks are members of a single group, granite. The specialized vocabulary of petrography covering them does not denote rocks which differ from granite; rather it denotes rocks which are species, subspecies, or varieties of granite. A symbolic classification that attempts to give more weight to geological and experimental information now available has been submitted elsewhere for publication.

#### MODAL COMPOSITION OF THE MAJOR MEMBERS OF THE SOUTHERN CALIFORNIA BATHOLITH

Modal analyses of specimens from the southern California batholith, collection of which was reported last year, have now been completed. The southern California batholith is one of the best-described rock masses of major dimensions. Previous workers have delineated four major petrographic members—granodiorite, hornblende tonalite, pyroxene tonalite, and gabbro. Most of the granodiorite has been mapped under the name of Woodson Mountain; the gabbro takes its name from the San Marcos Mountains, where it is best developed. Many geographic variants of the tonalites have been recognized, but petrographically all belong to one or other of the mineralogical types named above.

The hybrid nature of most of the tonalites is strongly evident in outcrop and frequently in hand specimen as well; they are

streaky, patchy aggregates consisting of lenses or remnants of an earlier-crystallized rock of high color index enclosed in or strewn through a quartz-rich feldspathic matrix. One of the largest known developments of dioritic rocks, the relation they establish between the gabbro and granodiorite of the region is a matter of primary petrological interest.

New modal analyses, some of which will be published elsewhere, strongly support the notion that their bulk composition has been established by a mechanical mixing of previously solidified gabbro with granodiorite magma. This mixing has provided an opportunity for reaction between magma and inclusions by means of which magmatic potassium that would otherwise have entered feldspar combines with magnesium and iron of the inclusions (probably in the pyroxene of the inclusions) to form biotite.

Despite an over-all inverse variation between quartz and color index, to the latter of which biotite is the major contributor, biotite itself varies *directly* with quartz in rocks of low color index. The relation between quartz and biotite is shown in figure 36. The line has been calculated only for specimens taken from rocks previously mapped as gabbro; from the symbols, however, it appears that a similar relation holds also for pyroxene tonalites, which are usually considerably poorer in quartz than hornblende tonalites. In the granodiorite and hornblende tonalites, on the other hand, the variation between biotite and quartz is inverse. Rocks of intermediate quartz content, the tonalites, contain considerably more biotite than those either rich or poor in quartz. As table 14 shows, potash feldspar, which is a major constituent of the granodiorite, is almost as low in the tonalites as in the gabbro.

The marked concentration of biotite in the intermediate rocks does not mask the general inverse variation of color index with quartz, but the elimination of biotite considerably strengthens this tendency. Figure 37 is a plot of quartz versus the

quantity (color index—biotite). Nearly 90 per cent of the total variance of either variable may be regarded as a linear function of the other. In rocks as coarse as this, sampling variation and analytical error could easily account for the 10.2 per cent of residual variance. As important as the

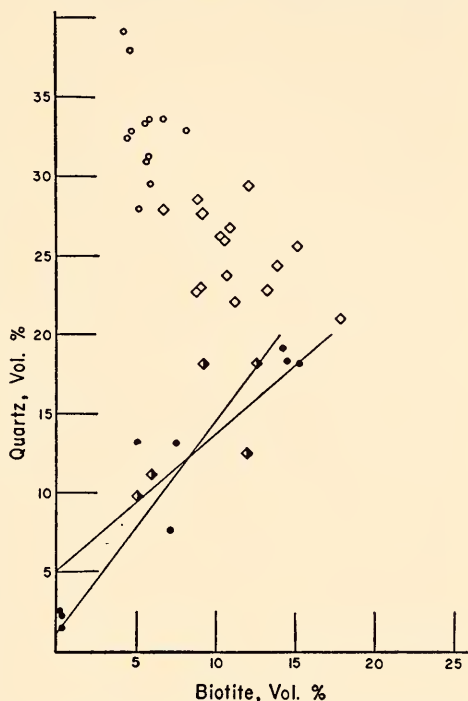


FIG. 36. Variation of quartz and biotite in some specimens from the southern California batholith. Key to symbols: open circle, Woodson Mountain granodiorite; open diamond, hornblende tonalite; half-solid diamond, pyroxene tonalite; solid circle, San Marcos gabbro. Each point is the average for two large thin sections cut from the same hand specimen.

high correlation is the slope of the regression line,  $-0.999$  if quartz is taken as dependent variable,  $-0.899$  if it is independent. If a quartz-free gabbro and a granodiorite devoid of hornblende, pyroxene, and ores were mechanically mixed in varying proportions, as by the injection of one into the other, all the resulting products would lie along a line of slope  $-1$ . The calculated errors of the regression coeffi-

TABLE 14  
AVERAGE COMPOSITIONS, MAJOR ROCK TYPES OF THE SOUTHERN CALIFORNIA BATHOLITH  
(VOLUME PER CENT)

	1	2	3	4	5
Quartz .....	32.0	33.4	25.2	14.0	10.7
Potassium feldspar .....	27.0	15.0	3.8	0.4	0.1
Plagioclase .....	34.9	44.0	51.4	58.4	58.2
Muscovite .....	<0.1	...	...	...	...
Biotite .....	5.3	5.7	11.3	9.0	7.3
Amphibole .....	...	1.2	7.5	8.3	11.8
Pyroxene .....	...	...	<0.1	8.8	9.5
Opaque .....	0.5	0.3	0.1	0.9	2.1
Nonopaque .....	0.3	0.4	0.6	0.1	0.2
Color index .....	6.1	7.6	19.6	27.1	30.8
Total feldspar .....	61.9	59.0	55.2	58.8	58.3

1. Adamellite, Santee and Simpson quarries, 4 specimens.
2. Woodson Mountain granodiorite, 12 specimens.
3. Hornblende-biotite tonalite, 15 specimens.
4. Pyroxene tonalites, 5 specimens.
5. San Marcos gabbro, 9 specimens.

cients are 0.12 and 0.13; the observed values are thus such as might well have been drawn from a parent population in which the true slope was  $-1$ .

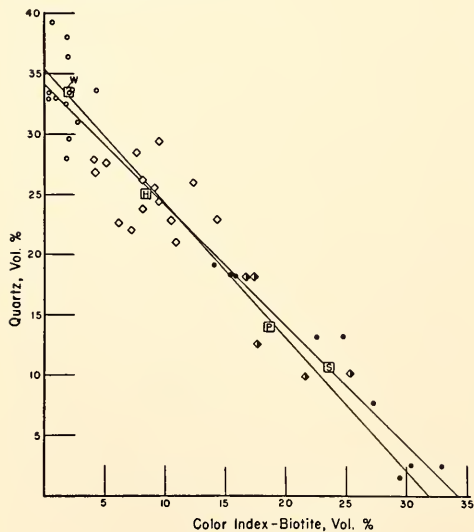


FIG. 37. Variation of quartz and (color index—biotite) in some specimens from the southern California batholith. Specimens and symbols as in figure 36. Average values indicated by W, Woodson Mountain granodiorite; H, hornblende tonalite; P, pyroxene tonalite; S, San Marcos gabbro.

It is of course possible to imagine other processes that might lead to an inverse variation between these two variables, but the variation would not ordinarily be linear and, if it were, its slope would not necessarily be unity. The hypothesis of hybridism implies such a relation for any pair of variables each of which is present in one "end member" and absent from the other. It has the further advantage of compatibility with a striking and prominent aspect of the field relations, something noted by previous students of the area.

Similar hybridism, characterized by intimate but incomplete mechanical mixing which fixes bulk composition and sets the stage for mineralogical reactions, has been proposed in connection with many small dioritic complexes, notably in the British Isles, and has even been suggested as a method by which lavas giving rise to the comparatively rare micaceous andesites might be generated. It does not seem to have been seriously suggested that hybridism of this sort might take place on a batholithic scale, fixing the petrography and bulk composition of rocks of a complex extending over many hundreds of square miles.



## SYMPOSIA

The enormous postwar expansion in the numbers of those engaged in scientific effort has profoundly altered human relationships among scientists. The annual national meetings of the various societies still have useful values, but their character has changed. The personal contacts, the vigorous discussions, the mutual stimuli of keen minds have been diluted by sheer numbers of people and simultaneous sessions. The subjects discussed by any one group have become limited to a narrow area, with the result that specialists talk only to specialists.

Many of these difficulties can be overcome by symposia in which a small selected group meets to discuss developments in an area of common interest. Subjects can be chosen that involve borderline fields. Attendance can include men of widely differing backgrounds and knowledge. The group can meet in pleasant, informal surroundings which exclude diverting influences. A general theme for the gathering is chosen in advance, but individual sessions need not be formally scheduled. As a result, discussion can center on what is new and challenging rather than on material that may be a year or more old.

During the past year the Geophysical Laboratory has been host to two such symposia. The enthusiasm of the participants at both events was high, and Professor Pettijohn of Johns Hopkins University summed up much of the opinion by stating that "this type of symposium solves the problem of scientific communication."

## VANNEVAR BUSH SYMPOSIUM

The Vannevar Bush Symposium on the Role of Volatiles in Geological Processes was held October 20-22, 1955, at Luray, Virginia, and in Washington, D. C. The meeting was arranged as a special tribute to Dr. Bush, who concluded on January 1, 1956, a long period of distinguished service as President of the Carnegie Institution of Washington. Working sessions were held

in Luray, with a final summary session taking the form of a formal dinner in Washington, at which Dr. Bush was guest of honor, attended by members of the symposium and other notables. The topic "Role of Volatiles in Geological Processes" was particularly appropriate for the occasion, since Dr. Bush actively fostered a new experimental approach to this area at the Geophysical Laboratory during his tenure as President. The subject was likewise a good choice for other reasons, but especially because investigations in this intrinsically important area are currently yielding many new and interesting results.

D. E. White opened the symposium with a discussion of the nature of the volatiles involved in geologic processes. White reviewed the considerable data available on the composition of hot spring waters and suggested that the chemical data may permit us to find hot springs which draw their supply from rocks undergoing metamorphic changes at depth. W. F. Libby discussed his research on tritium. Of particular interest to the conference was the use of the concentration of tritium in distinguishing hot springs which have been contaminated by meteoric waters and the use of the decay of tritium as an indicator of the age of circulating ground water.

That important clues are offered by isotopic fractionation in geologic processes was further emphasized by H. B. Craig, who discussed variations in the concentration of deuterium and  $O^{18}$  in natural waters, and by I. I. Friedman, who pointed up the possible uses of the variable abundance of deuterium. Craig has shown that the isotopic composition of the hydrogen and oxygen of the hot spring waters in Steamboat Springs, Yellowstone Park, and other thermal areas either is similar to that in the meteoric waters in these areas or can be derived from the meteoric waters by evaporation. Craig also showed that the concentration of  $C^{13}$  and  $O^{18}$  in carbonates deposited by hot springs may be

used as a geologic thermometer. This last discovery will be of importance in analyzing the life cycle of hot-spring areas. Friedman's work has enabled him to show that much of the water in volcanic glasses has been picked up since their eruption. Friedman has also begun a study of the concentration of deuterium in hydrous minerals, and the application of this work to metamorphic and igneous problems was pointed out.

The role of volatiles in igneous processes was reviewed by O. F. Tuttle, H. S. Yoder, and J. Verhoogen. Tuttle discussed his work with N. L. Bowen on the granite-water system. In addition to the hydrothermal work, Tuttle has obtained a large amount of data on the mineralogy of natural granites. The combination of these approaches has greatly furthered our understanding of the conditions under which granite forms. Yoder outlined his recent study of the melting relations under water pressure in the system diopside—anorthite. This system is fundamental to a study of basalts. Yoder's work has been particularly rewarding because of his discovery of the large shift of the eutectic composition with increase in water pressure. The shift may have application to the problem of layered laccoliths. Verhoogen contributed a discussion of the thermodynamics of silicate melt-water solutions.

Recent extreme pressure studies were outlined by F. Birch, G. J. F. MacDonald, and G. C. Kennedy. Birch discussed the data obtained at the Dunbar Laboratory on the reaction  $\text{nepheline} + \text{albite} \rightleftharpoons \text{jadeite}$ . MacDonald contributed information on coesite and aragonite, and pointed out that many of the transitions to high-pressure phases seem to be found in a pressure-temperature range comparable to that which must be present at the Mohorovičić discontinuity. The possibility exists that this discontinuity represents a phase change rather than a chemical change. Kennedy presented his data on the system  $\text{Al}_2\text{O}_3\text{—SiO}_2\text{—H}_2\text{O}$ . The use of very high pressure in working

out this system has greatly furthered the ease of attainment of equilibrium.

J. B. Thompson, Jr., contributed a discussion of the thermodynamic method of handling water as a variable in metamorphic processes. He suggested that geologists might use the concept of humidity in a manner analogous to its use in meteorology. The theoretical treatment of this problem is far ahead of experimental work, and the problem offers a promising field for laboratory investigation.

H. P. Eugster and R. I. Harker discussed some recent experimental work on systems important in the study of metamorphic rocks. Harker's data on the system  $\text{MgO—CaO—SiO}_2\text{—CO}_2$  have helped to develop a quantitative understanding of the metamorphism of siliceous dolomites. Eugster's study of the solvus in the system muscovite—paragonite has followed on the heels of the discovery that paragonite is an important and common mineral in metamorphic rocks. This solvus will be valuable as a geologic thermometer.

K. B. Krauskopf discussed the solubility of amorphous silica in fresh and sea water. Amorphous silica dissolves to form monomolecular solutions of  $\text{H}_4\text{SiO}_4$ . This solubility is independent of pH for values of pH below 9.

W. W. Rubey discussed direct development of different compositions of magma by progressive fusion of materials of the earth's mantle. With the aid of three-dimensional models, G. W. Morey presented the different possible types of binary systems involving a crystalline solid and a volatile component.

This symposium aimed to provide the participants with the opportunity for extensive discussion of new ideas and new areas for research in petrology. It is the hope of our staff that the benefits of the discussions will be felt far beyond the circle of those who attended.

A formal dinner with Dr. Bush as honored guest was held at the close of the scientific session. On this occasion E. F. Osborn reviewed some of the earlier con-

tributions of the Geophysical Laboratory and summarized the highlights of the working sessions at Luray.

In addition to members of the staff of the Geophysical Laboratory the following participated in the working sessions at Luray: Francis Birch (Harvard University), Harmon B. Craig (Scripps Institution of Oceanography), I. Irving Friedman (U. S. Geological Survey), James Gilluly (U. S. Geological Survey), Julian R. Goldsmith (University of Chicago), R. Ian Harker (Pennsylvania State University), Harry H. Hess (Princeton University), Earl Ingerson (U. S. Geological Survey), George C. Kennedy (University of California at Los Angeles), Konrad B. Krauskopf (Stanford University), Willard F. Libby (U. S. Atomic Energy Commission), Thomas S. Lovering (U. S. Geological Survey), E. F. Osborn (Pennsylvania State University), William T. Pecora (U. S. Geological Survey), Rustum Roy (Pennsylvania State University), William W. Rubey (U. S. Geological Survey), James B. Thompson, Jr. (Harvard University), George Tunell (University of California at Los Angeles), O. Frank Tuttle (Pennsylvania State University), John Verhoogen (University of California), Aaron C. Waters (Johns Hopkins University), and Donald E. White (U. S. Geological Survey).

#### METAMORPHIC FACIES SYMPOSIUM

"Metamorphic Facies" was the subject of a symposium held at the Geophysical Laboratory March 20-22, 1956. Motivation for arranging another meeting so soon after the Bush Symposium arose spontaneously from many sources. In part it reflected the success of the first venture, in part the opportunity presented by the presence of C. E. Tilley at the Laboratory, and it recognized an important current topic of geological interest. The subject is active, controversial, and draws from field observation, laboratory investigation, and theoretical physical chemistry. The program began with an address by F. J. Turner at

a special meeting of the Petrologists' Club. He discussed the nature of the problem and its history, and presented the new evaluation of the metamorphic facies concept developed by Fyfe, Turner, and Verhoogen. The facies are divided by them into two groups, contact and regional. The contact metamorphic group includes four facies of different temperature and water pressure ranges, in all of which load pressure (and hence the upper limit of water pressure) is low; water pressure commonly is less than load pressure. The regional metamorphic group consists of six facies of different temperature and pressure ranges, in all of which load and water pressure tend to be equal and are generally high.

The Symposium then undertook five intensive working sessions at which most of the leading workers in metamorphism were present. At the first of these, Tilley compared the perfection of zoning in the southern Scottish Highlands to the lack of systematic zoning in the Banffshire area. He cited new data on the change of composition of minerals with progressive metamorphism, particularly with regard to the change of muscovite composition at the common biotite isograd. He emphasized the need for more detailed mineralogical studies in the contact aureoles. A brief description of metamorphic zoning in the southern Alps of New Zealand was then presented by B. H. Mason. A unique reaction zone between dolerite and limestone in which pure zones of hydrogarnet, gehlenite, larnite, and scawtite were developed was also described by Mason. Many excellent examples of exsolution and replacement phenomena were presented by A. F. Buddington. His conclusions from these studies were applied to the granulite problem of the Adirondacks. Of particular value was the detailed description of the composition of feldspars, titaniferous magnetites, garnets, and amphiboles in those rocks of the eclogite, granulite, and amphibolite facies in which they occur.

In a following session H. Ramberg gave the results of his studies on the granulites



and amphibolites of Greenland. He specifically noted the presence of antiperthite, biotite, brown hornblende, and hypersthene in the granulites, and common hornblende, sphene, muscovite, and microcline in the amphibolites. J. B. Thompson, Jr., outlined his new method of plotting mineral assemblages. He assumes for pelitic rocks that quartz and muscovite are present and that equilibrium is reached at some externally controlled temperature, pressure, and activity of  $H_2O$ . Considering the components  $K_2O$ ,  $Al_2O_3$ ,  $FeO$ , and  $MgO$ , and projecting the compositions of various rock-forming minerals from the muscovite composition onto the  $Al_2O_3$ — $FeO$ — $MgO$  plane, he achieves a unique representation of the metamorphic assemblages.

In an evening session W. S. White lent support to the new zeolite facies by summarizing U. S. Geological Survey studies of mineral zoning in the Keweenaw lavas of Michigan. A. C. Waters detailed some of the problems connected with the zeolitic alteration of the lavas of Oregon and Washington. The new experimental results on Bowen's decarbonation series were then presented by R. I. Harker. In addition, Harker gave preliminary results on the effect of an inert gas on a system involving the breakdown of  $MgCO_3$ . Under a  $CO_2$  pressure less than the total pressure, a distinct lowering of the equilibrium temperature was observed.

Another session opened with remarks by M. P. Billings on the problems involved in the mapping of metamorphic zones. He was particularly concerned with the gneisses, and pointed up the difficulties with them on the new geologic map of New Hampshire that he compiled. The metamorphism of rocks high in magnesia was discussed by H. H. Hess. His observations on serpentine indicated that the process of serpentinization is haphazard and not related to migration of water from contacts. Serpentinization usually disappears with increasing metamorphism, and the rocks then contain amphibole, chlorite, and talc. Hess described some of the con-

tact metamorphic effects of a dunite on a green schist and the effects on a gabbro which cut a serpentine body.

The final session began with an account of the metamorphic zoning in northern Michigan by H. L. James. Some principal conclusions were that throughout regional metamorphism the  $FeO/Fe_2O_3$  ratio of a given bed in the iron-rich rocks is maintained, and that, although there was extensive redistribution of  $H_2O$ , there probably was no net increase or decrease for the region as a whole. G. J. F. MacDonald then presented an analysis and evaluation of the effect of stress on mineral stability and found that the effect is negligible. This was followed by a vigorous defense of A. Harker's stress mineral concept by Tilley. The symposium was closed with brief notes by R. I. Harker on the contact metamorphism at Carn Chuinneag, Scotland, and by A. Poldervaart on the metamorphism of a dike swarm south of Roan Mountain, North Carolina.

In addition to members of the staff of the Geophysical Laboratory the following participated: L. Thomas Aldrich (Department of Terrestrial Magnetism), Charles A. Anderson (U. S. Geological Survey), M. P. Billings (Harvard University), A. F. Buddington (Princeton University), J. D. H. Donnay (Johns Hopkins University), John W. Graham (Department of Terrestrial Magnetism), R. Ian Harker (Pennsylvania State University), Harry H. Hess (Princeton University), C. A. Hopson (Johns Hopkins University), Vernon J. Hurst (Georgia State Geological Survey), Harold L. James (U. S. Geological Survey), Brian H. Mason (American Museum of Natural History), E. F. Osborn (Pennsylvania State University), William T. Pecora (U. S. Geological Survey), Francis J. Pettijohn (Johns Hopkins University), Arie Poldervaart (Columbia University), Hans Ramberg (University of Chicago), Edwin W. Roedder (U. S. Geological Survey), William W. Rubey (U. S. Geological Survey), David B. Stewart (U. S. Geological Survey), James B. Thompson, Jr. (Har-



vard University), C. E. Tilley (Cambridge University), Francis J. Turner (University of California), O. Frank Tuttle (Pennsylvania State University), Aaron C. Waters (Johns Hopkins University), George W. Wetherill (Department of Terrestrial Magnetism), and Walter S. White (U. S. Geological Survey).

#### GEOCHEMISTRY COLLOQUIA AT JOHNS HOPKINS UNIVERSITY

The Geology Department at Johns Hopkins University and the Geophysical Laboratory participated in a rather unusual experiment in education during the spring semester of 1956. Each week a graduate seminar sponsored by the Laboratory was conducted at the University. The general theme was "Current Topics in Geochemistry and Experimental Petrology." Each speaker submitted in advance an abstract of his talk together with a selected bibliography. The students were thus enabled to examine background material before the

lecture. The experiment was a success. Both the speakers and the audience were enthusiastic about the stimulus experienced, and it is anticipated that an expanded series will be given later.

Topics and speakers included:

- "Dry silicate systems," by J. F. Schairer.
- "Alkali feldspars," by W. S. MacKenzie.
- "Plagioclases," by J. R. Smith.
- "Systems with water as a component," by G. W. Morey.
- "Amphiboles," by F. R. Boyd, Jr.
- "Micas and geological thermometry," by H. P. Eugster.
- "Sulfide systems," by G. Kullerud.
- "Thermodynamics in geology," by G. J. F. MacDonald (Massachusetts Institute of Technology).
- "The gabbro-granite relation in the southern California batholith," by F. Chayes.
- "Eh-pH relationships," by H. T. Evans (U. S. Geological Survey).
- "Paleobiochemistry," by P. H. Abelson.
- "Age determinations," by L. T. Aldrich (Department of Terrestrial Magnetism).

#### MISCELLANEOUS ADMINISTRATION

##### PETROLOGISTS' CLUB

The Petrologists' Club, now in its forty-sixth year, met on six evenings at the Laboratory this winter and spring. The club has grown to 160 active members. Interest in these meetings has always been great, and on several occasions this year the Laboratory's facilities were barely adequate to the attendance. The subjects discussed ranged over the fields of crystallography, petrology, and geochemistry.

The following papers were presented:

- "Screw dislocations and crystal growth," by W. Dekeyser (University of Ghent).
- "Heavy minerals, mineral stability, and tectonism," by F. J. Pettijohn (Johns Hopkins University).
- "Crystal chemistry of some vanadium minerals," by H. T. Evans (U. S. Geological Survey).
- "Hawaiian basalts," by H. A. Powers (U. S. Geological Survey).
- "Metamorphic facies," by F. J. Turner (University of California).

"Composition and significance of fluid inclusions," by E. W. Roedder (U. S. Geological Survey).

##### SEMINARS

The Laboratory has conducted a weekly series of seminars, with papers presented largely by staff members and concerned mainly with discussions of work in progress. Several talks were presented by outside speakers. These included the following:

- "Chemical equilibrium in metasomatic processes," by J. B. Thompson, Jr. (Harvard University).
- "Crystal-energy relations in silicates," by H. Ramberg (University of Chicago).
- "An equation of state for silicates at high pressure," by G. J. F. MacDonald (Massachusetts Institute of Technology).
- "Pre-Cambrian geology of the Fenno-Scandinavian Shield," by J. A. O. Kouvo (Princeton University).
- "Mineral zoning and ore transport," by H. L. Barnes (Columbia University).

"Some recent results of high-pressure investigations and some problems of terrestrial temperature," by S. Clark (Harvard University).

"Piezobirefringence in diamond," by E. Poindexter (University of Michigan).

"Theory of phase transitions," by T. Berlin (Johns Hopkins University).

"Synthetic basalts," by C. E. Tilley (Cambridge University).

"Correlation of electron motion in molecules," by K. S. Pitzer (University of California).

"Carbonate systems," by O. F. Tuttle (Pennsylvania State University).

"Uranium-lead ages," by G. W. Wetherill (Department of Terrestrial Magnetism).

"Isotopic composition of lead in ultramafics," by G. R. Tilton (Department of Terrestrial Magnetism).

"Rapakivi granites," by D. B. Stewart (U. S. Geological Survey).

"Development of gneissic structure in the Adirondacks," by P. Bartholome (Princeton University).

#### LECTURES

During the report year staff members were invited to present lectures as follows:

P. H. Abelson lectured at the Gordon Research Conference on Chemistry and Physics of Isotopes, American Association for the Advancement of Science; the Biological Laboratories, Harvard University; the Geological Society of Washington; the Society of Economic Paleontologists and Mineralogists of the American Association of Petroleum Geologists; and the Naval Medical Research Institute. He also discussed "The first life" on the National Broadcasting Company's television series "Our world in space."

The "Summary of Published Work" below briefly describes the papers published in scientific journals during the report year. In addition, the following papers are now prepared for publication: P. H. Abelson, "Paleobiochemistry"; F. Chayes, "A manual of modal analysis"; F. Chayes, "A provisional reclassification of granite"; F. Chayes, "The Holmes effect and the

G. Donnay lectured at the Mineralogical Society of Philadelphia and at the Point-Group Seminar, Physics Department, Polytechnic Institute of Brooklyn. As United States member of the Commission on Crystallographic Teaching, she reported at the Madrid meeting of the International Union of Crystallography.

J. L. England addressed a group of Civil Defense officials of Alexandria on radiation detection devices.

H. P. Eugster lectured at the Journal Club of the Department of Geology, Johns Hopkins University.

G. Kullerud lectured at the Symposium on the Associations and Genesis of Rare and Alloy Metals of the American Institute of Mining and Metallurgical Engineers.

G. W. Morey served as Visiting Professor at the Department of Geology and the Institute of Geophysics, University of California at Los Angeles, for two weeks in April. During this period he gave a series of five lectures on thermodynamics before a graduate seminar. He also lectured at the joint meeting of the American Institute of Mining and Metallurgical Engineers and the Society of Economic Geology.

J. F. Schairer lectured at the Eighth Annual Convention of the American Federation of Mineralogical Societies. He also delivered the 1956 Orton Memorial Lecture, "Melting relations of the common rock-forming oxides," at the annual meeting of the American Ceramic Society.

H. S. Yoder lectured at the Conference on Theoretical Geophysics sponsored by the National Science Foundation and the Department of Terrestrial Magnetism.

lower limit of modal analysis"; G. Donnay, F. C. Kracek, and W. R. Rowland, "The chemical formula of empressite"; W. F. Libby, "Relation between energy and half-thickness for absorption of beta radiation"; G. W. Morey and W. T. Chen, "Pressure-temperature curves in some systems containing water and a salt"; J. F. Schairer, "Melting relations of the common

rock-forming oxides"; J. R. Smith and H. S. Yoder, "Variations in X-ray powder diffraction patterns of plagioclase feldspars."

### SUMMARY OF PUBLISHED WORK

- (1232) Role of water in metamorphism. H. S. Yoder. Geol. Soc. Amer. Special Paper 62, pp. 505-524 (1955).

The role of water in metamorphism is determined by the independent variables rock pressure, temperature, water pressure, and the amount of water present. An analysis of the possible events in the regional metamorphism of an argillaceous sediment indicates that, if water flow takes place by way of pore spaces connected to the surface, the water vapor pressure may be very low and in the extreme case may approach that at the surface. If the pore spaces are isolated, the water pressure may be equal to or slightly greater than the rock pressure, depending on the strength and permeability of the rock. The mechanics of a representative metamorphic reaction are outlined under the two extreme conditions of water pressure with different flow rates and reaction rates. Water pressures lower than the rock pressure cause metamorphic reactions to run at lower temperatures. Experiments have been made under water pressures less than the total pressure. Differences in the water pressure from rock to rock may produce reversals in the normal sequence of isograds.

Independent of the water pressure, the water content has a great influence on the metamorphic assemblage. Since all bulk compositions including water as a component must be represented at each condition of rock pressure and temperature, it is possible according to Schreinemaker's principles to have at the same rock pressure and temperature representatives of several facies. The coexistence of eclogite, amphibolite, and green schist is cited as an example in which the mineralogy may be dependent solely on the water content. There are objections to the concept of the ubiquitous occurrence and adequate supply of water as a free phase in metamorphism.

The behavior of almandine garnets in "wet" and "dry" contact aureoles and in regionally metamorphosed rocks is developed in the light of the principles presented and of new experimental data. Some sequences of mineral assemblages may be explained equally well on the basis of a rate process involving variation

of the water pressure and on the basis of a static process involving variation of water content. This arises from the fact that at constant porosity (volume) and temperature the water content is proportional to the water pressure.

- (1234) Heterogeneous equilibria and phase diagrams. J. F. Schairer. *Ann. Rev. Phys. Chem.*, vol. 6, pp. 45-70 (1955).

More than 500 papers in the phase-equilibrium field are noted, and specific reference is made to 341 papers in this review for the year 1955. Graphical methods, theoretical studies, review articles, apparatus and methods, aqueous salt systems, organic systems, anhydrous salt systems, metal systems (including sulfides), and oxide, silicate, and refractory systems are covered in this review paper, which is one chapter in the *Annual Review of Physical Chemistry*.

- (1236) Precession goniometry to identify neighboring twins. G. Donnay, J. D. H. Donnay, and V. J. Hurst. *Acta Crystallogr.*, vol. 8, pp. 507-509 (1955).

On an orientation film taken with the precession camera, the normal to the reciprocal-lattice plane which is being photographed appears at the center of the limiting "circle" (given by the intense cutoffs of the white radiation streaks). If two reciprocal nets are nearly parallel, as can happen in twins, two limiting "circles" appear, and the distance between their centers can be converted into the angle between the normals to the two nets. The measurements can be made with sufficient accuracy ( $\pm 3'$ ) to discriminate between twin laws that could not otherwise be distinguished.

- (1237) The alkali feldspars: I. Orthoclase microperthites. W. S. MacKenzie and J. V. Smith. *Amer. Mineralogist*, vol. 40, pp. 707-732 (1955).

A study of the lamellar structure of a number of analyzed feldspars belonging to the orthoclase-microperthite series has been made using X-ray oscillation photographs. Differences in the X-ray diffraction patterns have



been correlated with the bulk composition of the feldspars. Measurements of the reciprocal lattice angles  $\alpha^*$  and  $\gamma^*$  of the soda phase give results indicating that the composition of the soda phase may be determinable from these angles.

The variations in optic axial angle for a known chemical composition have also been correlated with differences in the X-ray diffraction patterns.

- (1238) The alkali feldspars: II. A simple X-ray technique for the study of alkali feldspars. J. V. Smith and W. S. MacKenzie. *Amer. Mineralogist*, vol. 40, pp. 733-747 (1955).

A routine X-ray method has been developed for the study of alkali feldspars. From a single-crystal X-ray oscillation photograph the symmetry, twinning, and unmixing can be seen at a glance. The reciprocal lattice angles  $\alpha^*$  and  $\gamma^*$  of triclinic phases, twinned on either the albite or the pericline law, can be readily measured from the oscillation photograph by a new precision method.

- (1239) Modal composition of two facies of the Carnmenellis granite. F. Chayes. *Geol. Mag.*, vol. 92, pp. 364-366 (1955).

Modal analyses of nine specimens of Ghosh's Type I and six specimens of his Type II Carnmenellis granite indicate that there is no significant difference between the average compositions of these two facies.

- (1240) The system  $K_2O-Al_2O_3-SiO_2$ . J. F. Schairer and N. L. Bowen. *Amer. Jour. Sci.*, vol. 253, pp. 681-746 (1955).

Results of quenching experiments at temperatures between the liquidus and that of complete consolidation are reported for 383 synthetic compositions. These locate the liquidus surfaces and the fields of the primary phases cristobalite, tridymite, quartz, mullite, corundum, potash feldspar, leucite, hexagonal  $KAlSiO_4$ , orthorhombic  $KAlSiO_4$ , potassium tetrasilicate, and potassium disilicate. The temperatures and compositions of ten binary and eleven ternary invariant points within the system  $K_2O-Al_2O_3-SiO_2$  were located, and an equilibrium diagram for this ternary system is given. The subsystems leucite—corundum— $KAlSiO_4$ , leucite—corundum—silica, leucite— $KAlSiO_4$ —potassium disilicate,

and leucite—potassium disilicate—silica are ternary systems within the larger ternary system  $K_2O-Al_2O_3-SiO_2$ . No field of " $\beta-Al_2O_3$ " was found, and, whatever may be its composition, " $\beta-Al_2O_3$ " is metastable with respect to corundum at the temperatures investigated. Our knowledge of the stability relations of the polymorphs of  $KAlSiO_4$  (kaliophilite, kalsilite, and orthorhombic  $KAlSiO_4$ ) is still very unsatisfactory, and this matter requires further study. Dry melts of feldspar and silica which approach a granitic composition can dissolve little aluminous material unless the temperature is very high.

- (1241) The system  $NaPO_3-Na_4P_2O_7-K_4P_2O_7-KPO_3$ . G. W. Morey, F. R. Boyd, Jr., J. L. England, and W. T. Chen. *Jour. Amer. Chem. Soc.*, vol. 77, pp. 5003-5011 (1955).

This is a study of the phase-equilibrium relations in that part of the ternary system  $Na_2O-K_2O-P_2O_5$  included within the limits  $NaPO_3-Na_4P_2O_7-K_4P_2O_7-KPO_3$ . The binary system  $Na_4P_2O_7-K_4P_2O_7$  shows a complete series of solid solutions with a minimum melting point. All compositions on the join  $Na_5P_3O_{10}-K_5P_3O_{10}$  melt incongruently, there probably is a compound of the composition  $Na_5-P_3O_{10} \cdot K_5P_3O_{10}$ , and there is extensive solid solution. The fields of these solid solutions series have been determined, as well as those of the compounds  $NaPO_3$ ,  $3NaPO_3 \cdot KPO_3$ , and  $KPO_3$ .

- (1242) The temperature of deposition of sphalerite-bearing ores in the Caledonides of northern Norway. G. Kullerud, P. Padgett, and F. M. Vokes. *Norsk Geol. Tidsskrift*, vol. 35, pp. 121-127 (1955).

Six such deposits were carefully sampled. The sphalerite in each sample was separated from the other ore minerals and analyzed for iron, manganese, and cadmium. Specimens from five of the deposits showed free pyrrhotite. The temperatures of formation of these sphalerites as determined by application of the sphalerite geothermometer were found to lie within a narrow range of  $515^\circ \pm 40^\circ$  C.

Specimens from the sixth deposit contained no free iron sulfide, and all the temperatures obtained were  $50^\circ$  to  $75^\circ$  C lower than those obtained from specimens from the first five deposits.



The results support the idea of a uniform mineralization in the Caledonide schists of northern Norway, as deduced from the assemblage of ore minerals and from the mode of occurrence of the ore bodies.

- (1243) Synthetic and natural muscovites. H. S. Yoder and H. P. Eugster. *Geochim. et Cosmochim. Acta*, vol. 8, pp. 225-280 (1955).

The syntheses of randomly stacked one-layer monoclinic (1Md), one-layer monoclinic (1M), and two-layer monoclinic (2M) muscovite have been accomplished. The upper stability limit of muscovite is represented by a curve passing through the points 625° C, 5000 psi water pressure; 665° C, 15,000 psi; and 715° C, 30,000 psi. Immediately above this curve, sanidine + corundum + vapor are the stable phases.

The stability ranges of the muscovite polymorphs named could not be fixed accurately because of the sluggish nature of the transformations; however, the transformation of 1Md → 1M → 2M was effected. It is believed that this sequence obtains in the progressive metamorphism of a sediment. The first transformation is probably dependent on factors affecting reaction rate. The second transformation may be related to a univariant curve of equilibrium (i.e. an isograd). The growth of a three-layer trigonal muscovite (3T) was suspected in only one run with 2M muscovite, and its synthesis is therefore not substantiated.

Study of natural muscovites and muscovite-like materials indicates that the hitherto unrecognized 1Md and 1M muscovites are common. Their relation to materials called illite, high-silica sericite, and hydromuscovite, as well as to other dioctahedral minerals, is described.

The complete destruction of the muscovite structure by grinding is demonstrated by selected area electron diffraction studies. The alleged resynthesis of muscovite by further grinding is shown to be suspect.

A comparison of the maximum upper stability curve of muscovite with the minimum-melting curve of the "granite" system suggests that muscovite may form in granitic magmas above approximately 1500 atmospheres water pressure, and in the solid state below that pressure in granitic rocks when the water pressure equals the total pressure. These relations may account for the apparent two gen-

erations of muscovite in some granites. A limitation on the amount of muscovite in igneous granites is believed to be imposed by the nature of the liquidus surfaces.

The upper stability curve of muscovite marks the maximum possible conditions of the second sillimanite or orthoclase isograd in metamorphic rocks.

The nature of the processes of weathering, abrasion, and diagenesis of muscovite in sediments is outlined.

- (1244) The action of hot water on some feldspars. G. W. Morey and W. T. Chen. *Amer. Mineralogist*, vol. 40, pp. 996-1000 (1955).

Hot water was passed over the feldspars albite and orthoclase contained in closed bombs, the total amount of solution weighed and analyzed, and the material remaining in the bomb studied with X rays and the petrographic microscope. At 350° C, 5000 psi, the solutions obtained from orthoclase and from albite contained over 90 per cent of the dissolved material in the feldspar ratio. At lower temperatures and pressures, 200° C and 2000 psi, and 100° and 40 psi, albite was decomposed in greater proportion. At 350° and 5000 psi a large amount of analcite was found in the albite experiment, but none was found at the lower temperatures.

- (1245) Analysis of Hamley bornite from South Australia. E. G. Zies and H. E. Merwin. *Amer. Mineralogist*, vol. 40, pp. 1001-1003 (1955).

The physical and chemical analysis of a bornite from the Hamley Mine, Moonta, South Australia, is given. The composition of the selected sample conforms very closely indeed to that demanded by the formula  $\text{Cu}_5\text{FeS}_4$ .

- (1246) The system  $\text{Na}_2\text{O}-\text{Al}_2\text{O}_3-\text{SiO}_2$ . J. F. Schairer and N. L. Bowen. *Amer. Jour. Sci.*, vol. 254, pp. 129-195 (1956).

Results of quenching experiments at temperatures between the liquidus and that of complete crystallization are reported for 340 synthetic compositions. These locate the liquidus surfaces and the fields of the primary phases cristobalite, tridymite, quartz, mullite, corundum, albite, nepheline, carnegieite, sodium disilicate, sodium metasilicate, and sodium orthosilicate. Some of these solid phases

are of variable composition because of solid solution. The temperatures and compositions of the many binary and ternary invariant points within the system  $\text{Na}_2\text{O}-\text{Al}_2\text{O}_3-\text{SiO}_2$  were located, and an equilibrium diagram for this ternary system is given. The six subsystems albite—corundum—silica, albite—nepheline—corundum, carnegieite— $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3$ —corundum, albite—sodium disilicate—silica, albite—nepheline—sodium disilicate, and nepheline—sodium disilicate—sodium metasilicate are ternary systems within the larger ternary system  $\text{Na}_2\text{O}-\text{Al}_2\text{O}_3-\text{SiO}_2$ . The carnegieites in the quadrilateral  $\text{Na}_2\text{SiO}_3-\text{NaAlSiO}_4-\text{NaAlO}_2-\text{Na}_4\text{SiO}_4$  are ternary solid solutions between  $\text{NaAlSiO}_4$ ,  $\text{NaAlO}_2$ , and  $\text{Na}_2\text{SiO}_3$ . No field of " $\beta\text{-Al}_2\text{O}_3$ " was found, and, whatever may be its composition, it is metastable with respect to corundum at the temperatures investigated. The data presented here for the system  $\text{Na}_2\text{O}-\text{Al}_2\text{O}_3-\text{SiO}_2$  are of fundamental importance in depicting the crystallization behavior of the two ternary compounds, the soda feldspar albite and the feldspathoid nepheline, with all possible excesses of the component oxides, and in depicting the compatibilities, or lack thereof, of the various solid phases. Some of the many petrologic and technologic applications of the data are indicated.

- (1247) The orientation of the pericline twin lamellae in triclinic alkali feldspars. W. S. MacKenzie. *Mineralogical Mag.*, vol. 31, pp. 41-46 (1956).

Crosshatched twinning is characteristic of two alkali-feldspar species, microclines and anorthoclases. Because of the difference in the crystallographic angles of these two species, the crosshatched twinning is most clearly seen in (001) sections of microcline and in sections perpendicular to the *a* crystallographic axis in anorthoclases. If pericline twinning is present it is generally seen in (010) sections of microclines, but if the microcline is microperthitic the twin lamellae may not be seen. Strings or lamellae of perthitic albite can be distinguished from pericline twin lamellae by refractive-index differences and also by the angle that their trace makes with the trace of (001) in (010) sections.

- (1248) Annual report of the Director for 1954-1955.

- (1249) The powder patterns and lattice parameters of plagioclase feldspars. I. The soda-rich plagioclases. J. V. Smith. *Mineralogical Mag.*, vol. 31, pp. 47-68 (1956).

Geiger-counter powder records of soda-rich plagioclases have been carefully measured and indexed. The lattice parameters of the synthetic specimens are almost independent of the lime content, the largest variation (that in  $\beta^*$ ) amounting to only  $0.2^\circ$ . After strong heating the powder patterns of the natural specimens closely approached those of the synthetic specimens.

Eight out of the nine natural plagioclases gave lattice parameters which fall within experimental error on a continuous line. There is a fairly large variation in lattice parameters, especially for  $\gamma^*$ , which changes by  $2^\circ$ . As the lime content increases, the lattice parameters of the natural plagioclases approach those for the high-temperature synthetic and heated natural specimens until at  $\text{An}_{50}$  the separation is only one-fifth of that at  $\text{An}_0$ . The ninth specimen (from a dacite) gives parameters midway between the curves, thus exhibiting a state of partial inversion.

The partially heated natural plagioclases and the plagioclase from the dacite give parameters indistinguishable within experimental error from the parameters of unheated plagioclases with a higher lime content. There is, therefore, no reliable powder X-ray method for the determination of composition in the region  $\text{An}_0$  to  $\text{An}_{50}$ . If the composition is known, the powder method may be used for the determination of the thermal state. If the plagioclase can be judged from geological evidence to be in the low-temperature state, the powder method can be used to estimate the composition with an accuracy of 2 per cent  $\text{An}$ .

- (1250) Staurolite twinning. V. J. Hurst, J. D. H. Donnay, and G. Donnay. *Mineralogical Mag.*, vol. 31, pp. 145-163 (1956).

The space group *Ccmm* of the accepted staurolite structure ( $7.82 : 16.52 : 5.63 \text{ kX} = 0.473 : 1 : 0.341$ , Náray-Szabó, 1929) is a pseudo space group; the true one has no *c* glide plane. Staurolite is monoclinic pseudo-orthorhombic, as is shown optically by horizontal dispersion and morphologically by unequal developments of *r* (201) and *r'* ( $\bar{2}01$ ).

It has variable cell dimensions,  $7.83\text{--}7.95 : 16.50\text{--}16.82 : 5.62\text{--}5.71$  Å, constant  $\beta = 90^\circ \pm 3'$ , aspect  $C^*$ , and probable space group  $C2/m$  (negative pyroelectric test). This leads to the prediction of a new type of twin, "by high order merohedry," a penetration twin that simulates an orthorhombic single crystal. It is known (Friedel, 1922) that the  $90^\circ$  cross can be accounted for by two different twin laws, and the  $60^\circ$  cross by five. The twin operations are respectively the pseudosymmetry operations of a pseudotetragonal cell, obtained by transformation  $010/003/100$ , and those of Mallard's pseudo cube, resulting from transformation  $013/0\bar{1}3/300$ . By precession methods more than one twin law is established for each type of cross: both laws  $[100]_{90^\circ}$  and  $[013]_{180^\circ}$  are found for  $90^\circ$  crosses;  $[313]_{180^\circ}$  and  $[102]_{120^\circ}$ , for  $60^\circ$  crosses.

- (1251) The alkali feldspars. III. An optical and X-ray study of high-temperature feldspars. W. S. MacKenzie and J. V. Smith. *Amer. Mineralogist*, vol. 41, pp. 405-427 (1956).

A series of high-temperature alkali feldspars has been studied both optically and by X rays. Almost all the specimens appear under the microscope to be optically homogeneous, but X-ray studies show that those in the composition range  $\text{Or}_{60}\text{Ab}_{40}\text{--}\text{Or}_{25}\text{Ab}_{75}$  are unmixed to some extent. Single-crystal X-ray photographs of the unmixed specimens enable the separate phases to be studied, and in some cases the reciprocal lattice angles  $\alpha^*$  and  $\gamma^*$  of the sodium feldspar phase can be measured. The values obtained for these angles correspond well to those of single-phase, high-temperature sodium-rich feldspars.

The effect on the mean value of the optic axial angle of heating at  $900^\circ\text{C}$  for 24 hours has been studied; a possible discontinuity in the series near the composition  $\text{Or}_{60}\text{Ab}_{40}$ , which has not been previously noted, was revealed.

- (1252) Experimental geology. G. W. Morey. *Jour. Phys. Chem.*, vol. 60, pp. 718-724 (1956).

A major concern of geology is to explain the characteristic association of certain minerals in well known rock types, and the relation of these rock types to one another. From experimentally determined phase-equilibrium

diagrams it is shown how by a series of stages of fractional crystallization, many of which result in the formation of solid solutions, rocks high in silica, such as granite, can be derived from an initial basic magma low in silica from which orthosilicates such as the olivines are the primary crystallization. This process of separation of chiefly anhydrous minerals results in the accumulation in the magma of volatile constituents, chiefly water, which may result in the building-up of pressure, giving rise to a volcanic eruption; or the cooling process may be continuous, with the formation of pegmatites, fumaroles, and hot springs.

- (1253) Radioactive ages of minerals from the Brown Derby Mine and the Quartz Creek granite near Gunnison, Colorado. L. T. Aldrich, G. L. Davis, G. R. Tilton, and G. W. Wetherill. *Jour. Geophys. Research*, vol. 61, pp. 215-232 (1956).

Radioactive age determinations have been made on sixteen minerals from the Brown Derby pegmatite and the Quartz Creek granite. A high degree of consistency has been found among the Rb-Sr ages measured on micas and feldspars. A similar degree of consistency exists in the K-A ages of micas. These consistencies are indicative of the usefulness of measurements of the decay of  $\text{K}^{40}$  and  $\text{Rb}^{87}$  in determining relative ages of igneous rocks. U-Pb and Th-Pb ages determined from minerals obtained from the pegmatite and granite were not concordant. Different minerals showed different types of partial separation of parent and daughter elements. The measurements are most consistent with an age of  $1350 \pm 100$  million years for the pegmatite and the granite.

- (1254) The evolution of the igneous rocks. N. L. Bowen. New York, Dover Publications, Inc. (1956), a reprint edition of the original book published by Princeton University Press in 1928. x + 338 pp., 82 figs., with a new introduction by J. F. Schairer and a complete bibliography of the writings of N. L. Bowen.

This classic volume in chemical petrology, which has been out of print for several years and which is still in great demand, has just been reprinted. The book, first published in 1928, was based on a course of lectures given



to advanced students in the Department of Geology at Princeton University in the spring of 1927. This volume has had a profound influence on the younger generation of geologists because it emphasized the sound principles of physical chemistry that underlie geological processes. Although much additional information has been acquired both in the laboratory and in the field since the volume was first published, Bowen's application of the principles of physics and chemistry is as cogent today as it was then.

The chapter headings are as follows: I, The problem of the diversity of igneous rocks; II, Liquid immiscibility in silicate magmas; III, Fractional crystallization; IV, Crystallization in silicate systems; V, The reaction principle; VI, The fractional crystallization of basaltic magma; VII, The liquid lines of descent and variation diagrams; VIII, The glassy rocks; IX, Rocks whose composition is determined by crystal sorting; X, The effects of assimilation; XI, The formation of magmatic liquid very rich in potash feldspar; XII, The alkaline rocks; XIII, Lamprophyres and related rocks; XIV, The fractional resorption of complex minerals and the formation of strongly femic alkaline rocks; XV, Further effects of fractional resorption; XVI, The importance of volatile constituents; XVII, Petrogenesis and the physics of the earth; XVIII, The classification of igneous rocks.

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## DEPARTMENT OF PLANT BIOLOGY

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One group in the Department has continued to center its activities on the study of the mode of action of the pigments concerned in photosynthesis and their state of combination in live cells.

Many metabolic reactions of living matter and of its component enzyme systems have been studied by taking the systems apart into chemically characterizable units and then trying to recover the natural activity by putting the parts together again. This approach has not been widely used in investigations on the pigment part of the mechanism of photosynthesis. It has usually been found that if the pigments of chloroplasts are removed, the original photochemical activity cannot be restored by recombining the pigments with the extracted residue. There have, however, been several reports of limited success in obtaining some reactivation of one of the steps of the photochemical part of photosynthesis by means of the replacement of chlorophyll which had been removed from chloroplasts. Dr. Lynch has attempted to repeat such experiments, using what were considered to be improved test methods. Her experiments were entirely unsuccessful.

In the course of this work, however, it was found that one of the yellow pigments which generally accompany chlorophyll in green plants can be removed and later replaced in such a way as to give back a large part of the activity that had been lost by its extraction. Fragmented Swiss chard chloroplasts that had been dried at low temperature after freezing were used. The carotene was removed by cold extraction of the dry chloroplast material with petroleum ether. The measured activity of the chloroplast fragments was the reduction of a blue dye which takes place when the dry chloroplast powder is suspended in a water solution of the dye and illuminated. When the carotene was extracted

from the dry powder, the activity was reduced to about 10 or 20 per cent of normal. The activity came back to nearly normal when the petroleum ether extract, or a solution of pure  $\beta$ -carotene, was evaporated on the extracted powder. The dye-reduction activity test is a measure of the ability of the chloroplast material to use light for the formation of chemical reducing power. The reaction is very closely related to the way that chlorophyll functions in photosynthesis.

The experiments thus far have been exploratory and largely qualitative. Further work, and confirmation of the effect by other test systems, will be necessary before it can be concluded that  $\beta$ -carotene is specifically an active factor in normal photosynthesis. Nevertheless, these experiments have already shown a promising new way to study the long-standing question as to how the carotenoid pigments are connected with photosynthesis in green plants.

Another particularly interesting series of experiments was carried out by Dr. Shibata, a research fellow from Japan. He developed a simple method for recording the absorption spectra of small amounts of pigments in living leaves. Protochlorophyll, the precursor of chlorophyll, exists in minute quantities in leaves that have been grown in the dark. Previously the transformation of protochlorophyll to ordinary chlorophyll, a transformation which takes place rapidly when dark-grown leaves are illuminated, had to be studied either by fluorescence spectroscopy of the killed leaves or by subsequent extraction of the pigments from a number of leaves. With the new method it is possible to measure the complete absorption spectrum of protochlorophyll and of chlorophyll directly in the living leaf while the transformation is taking place. Thus the process of chlorophyll formation has now been followed di-

rectly in leaves. A previously unsuspected intermediate form of chlorophyll was found. The type of chlorophyll formed immediately upon illumination of the protochlorophyll in a dark-grown leaf has its maximum absorption peak at an appreciably longer wavelength (684 m $\mu$ ) than had been previously known. This type of chlorophyll then changes in about ten minutes into a form absorbing at 672 m $\mu$ , which is then more slowly converted to normal chlorophyll *a* with a peak absorption at 677 m $\mu$ . It was also found that chlorophyll in its final form when present in very small amounts in the leaves is itself sensitive to light in a way that may be directly related to the means by which chlorophyll *a* normally participates in the process of photosynthesis.

Further progress has been made by Drs. Smith and Kupke in their work on the purification and characterization of the protochlorophyll-protein complex obtained from dark-grown leaves. The ultracentrifugation procedures previously described are now being supplemented by fractional precipitation with salts. Active protochlorophyll is attached to proteinaceous particles, of uniform size and probably nearly spherical in shape, having an estimated molecular weight of about 400,000. Partially purified preparations of these particles may be frozen and dried without losing their ability to transform their protochlorophyll into chlorophyll *a* upon illumination. It has been known for some time that the transformation by light may take place in a normal leaf at temperatures far below freezing. Recently the equally extraordinary fact has been discovered that the frozen and dried partially purified particles may transform their protochlorophyll to chlorophyll when illuminated in the dry state just as readily as when dissolved in water. These active particles have been purified to the extent that the dialyzable components have been removed and there remain only two sedimentable components detectable by ultracentrifugation. By rough calculation of the areas under the peaks of

the analytical ultracentrifuge photographs, it has been estimated that the better preparations are approximately 75 per cent pure. A number of different methods are under study for their utility in further purification. Preparative ultracentrifugation and salt precipitation as well as zone electrophoresis have been investigated, and a number of the properties of the particles have been studied. So far, precise measurements of composition have not been undertaken because the material is not yet pure, but it is already evident that the amount of protochlorophyll in these particles is quite small in comparison with the amount of protein. This situation is in direct contrast to the findings of various laboratories on the composition of chlorophyll-protein complexes from mature leaves. With fully grown leaves the amount of chlorophyll in relation to the amount of protein present in the preparations is very much larger than can be explained by the formation of a simple compound between chlorophyll and its protein. This study of the chlorophyll-protein complex in its early stages of formation should in time contribute significantly to our still sparse knowledge of the chemical nature of chlorophyll as it occurs in the living leaf.

Dr. Bernard L. Strehler, a Visiting Investigator from the University of Chicago, correlated the light-induced changes of absorption in *Chlorella* with the ability of this alga to emit light. The correlation in time between "delayed light" production and the absorption changes in the live cells after an illumination period corroborates the idea that the light is produced at the expense of chemical substances that appear to be themselves products formed by photosynthesis. Some of the absorption changes were found to be reversed in sign, as compared with the changes in continuous light, when the algae were measured in a flowing system that gave a brief dark period after the illumination. The existence of previously known absorption bands sensitive to light at 480, 525, and 555 m $\mu$  was



confirmed, and new bands were found at 648 and 660 m $\mu$ .

Marine plankton swim in the ocean in a direction that is controlled by light. The colors of light that are responsible for this phototaxis of the dinoflagellates are not the same as those most effective in photosynthesis. Dr. Per Halldal, a Visiting Investigator from Oslo, has been measuring the effectiveness of light of different wavelengths in orienting the swimming of several dinoflagellates. Three different types of action spectra were found. The pigments responsible for them have not yet been identified and are not detectable in extracts of the organisms. So far, all that is known of these pigments that so critically determine the behavior of these plankton is the shape of their action spectra.

A large agar surface having a temperature gradient and a light-intensity gradient at right angles to each other has been used for the study of algal growth. With this device the growth of a single culture under different combinations of temperature and light intensity can be observed in a single experiment. The influence of intensity at various constant temperatures and of temperature at a series of constant intensities has thus been investigated for several common algae by Dr. Halldal.

A conference on genes, mechanisms, and expression of character, held in honor of Dr. Vannevar Bush on November 9 and 10, 1955, was attended by members of the staff and invited guests active in this field of biological research. Basic problems relating to current and future developments in plant biology were discussed. The spectrum of viewpoints represented in the biological fields, ranging from the one exemplified in experimental taxonomy to others concerned with physiological processes, biochemical genetics, and biochemistry was reviewed, and the role of the Department's program was considered in this connection. A conclusion reached was that a shift in emphasis from experimental taxonomy to physiological genecology is timely and desirable.

For the past year the major concern of the Experimental Taxonomy group in the Department has been to complete the manuscript for a book on the genetics of climatic races. The experiments with *Potentilla* (a native wild plant related to the strawberry) upon which the discussion of the general principles of the subject is based were started in the Department's gardens in 1932. The completion of this manuscript will bring to a close one of the major lines of study in which this group of investigators has been engaged since the establishment of the Department in its present location.

The next project of long standing whose conclusion seems possible in a few years is the study of range-grass genetics that resulted in the development of breeding methods suitable for the genetic control of so-called apomicts. Apomictic species are those which reproduce themselves primarily without fertilization by other plants. Such plants thus remain stable once the desired combination of characters has been attained. The range-grass work has two major objectives. The first is to prepare for publication findings relating to basic biological structure and the evolution of species in the bluegrass group; the second is to record the new breeding methods that have been found to be effective with this apomictic material in developing new strains of forage grasses.

Plantings of the selected grasses at the Institution's gardens have been supplemented by co-operative arrangements with our long-time collaborators in the U. S. Soil Conservation Service and by a more recent and widespread arrangement with numerous state groups co-ordinated by the U. S. Agricultural Research Service. Records of the plots for the next three years will complete the data for a description of the behavior of the new types of hybrid grasses in different environments. This form of collaboration will also help in introducing the new grasses to agriculture by making it feasible for various state and national agencies to release for use those strains

that are found to be of practical value for local conditions. The new plantings were studied this spring by Drs. Clausen and Hiesey in Virginia, Kentucky, Indiana, Pennsylvania, Louisiana, Missouri, Oregon, and California. Records were also made by Dr. Clausen and Mr. Nobs of both the newer and the older field tests at two stations in Oregon and two in Washington.

Among developments of the year in experimental taxonomy, two major aspects are worthy of special mention. One is the comparative study of fifteen apomictic lines derived from a single first-generation hybrid between *Poa ampla* from Kahlotus, Washington, and *P. pratensis* from the Athabasca region in Canada. Though the individual from which these lines originated was not apomictic itself, there were many apomictic strains in the second generation. Eight of the apomictic derivatives were selected among seedling progeny grown at Stanford, five from progeny growing at Pullman, and two at Edinburgh, Scotland. Studies on this entire series of apomicts established for one year at Stanford, Pullman, and Mather indicate a wide diversity in response patterns among the derivatives selected at Stanford, Pullman, and Edinburgh. Observations on some of these lines have also been made in plantings in the eastern and midwestern states. It is apparent that a very wide range of selection is possible among the

progeny of a single new sexually reproducing hybrid. This fact is of interest in interpreting the evolution of the complex genus *Poa*, and also in the practical development of forage grasses for agricultural uses.

Another development of major importance is the production for the first time of stable apomictic derivatives of the combination *Poa ampla* from the Palouse Prairie region and *P. arida* from Nebraska. The extreme vigor of the first-generation hybrids of this origin at Pullman and at Mather indicated the desirability of establishing stable apomictic lines from them. Among seventy-five such hybrids whose progeny are being tested, three appear to be sufficiently apomictic to warrant further study.

Production of seed of selected apomictic hybrid lines has been continued by the Soil Conservation Service nurseries at Pullman, Washington, and at Pleasanton, California, the principal seed sources for apomictic hybrid lines to be tested in field plots. The Ferry-Morse Seed Company has given the Department 180 pounds of seed of one of our apomictic lines of *Poa scabrella*  $\times$  *P. ampla* which appears to have potential value in the lowland ranges of California. A race of *P. pratensis* originating from the coast of Oregon has shown promise as a turf grass, and is now being grown by the Ferry-Morse company for possible commercial use.

## PERSONNEL

### BIOCHEMICAL INVESTIGATIONS

*Staff:* C. Stacy French, *Director*, Donald W. Kupke, Harold W. Milner, James H. C. Smith

*Visiting Investigators:* Per Halldal, Bernard L. Strehler

*Research Fellows:* Paul H. Latimer, Josef E. Loeffler, Kazuo Shibata

*Research Assistants:* Arthur T. Giese, Victoria H. Lynch

*Technical Assistants:* Ingrid M. Ahrne, Lois J. Durham, William L. Fairchild, Gordon E. Harper, Bruno F. Ludovici

### EXPERIMENTAL TAXONOMY

*Staff:* Jens C. Clausen,<sup>1</sup> William M. Hiesey

*Visiting Investigators:* Ursula Brodführer, Harlan Lewis, Chakravarti S. Venkatesh, George H. Ward

*Research Fellow:* F. J. F. Fisher

*Research Assistants:* Malcolm A. Nobs, Paul L. Pfahler, Thomas R. Pray

*Technical Assistants:* Lois M. Cox, John F. Hansen

<sup>1</sup> Retired June 30, 1956.

*Clerical Assistants:* Alberta B. Caswell, Helen D. Gill

*Gardener:* Wesley B. Justice

#### RESEARCH ASSOCIATE

Ralph W. Chaney, Professor of Paleontology,  
University of California, Berkeley

#### DEPARTMENT SECRETARY

Wilbur A. Pestell

#### MECHANICAL ENGINEER

Louis R. Kruger<sup>1</sup>

#### CUSTODIANS

Frank E. Russell,<sup>1</sup> Joseph H. Goss

Dr. Jens C. Clausen retired on June 30, 1956, after twenty-five years with the Institution. He continues to be an active participant in the completion of the undertakings in experimental taxonomy, most of which have involved the observation of many generations of plants over a long period of years.

Dr. Clausen served during the year as President of the Society for the Study of Evolution. Dr. William M. Hiesey served as President and Mr. Malcolm A. Nobs as Treasurer of the California Botanical Society. Dr. James H. C. Smith acted as a Director of the Santa Clara Valley Section of the American Chemical Society, and Dr. C. Stacy French served as President of the Society of General Physiologists.

Dr. F. J. F. Fisher, University of Melbourne, Australia, began studies on differences in growth response under various conditions of light and temperature in low- and high-altitude forms of the *Mimulus cardinalis* complex, with the purpose of probing into the physiological differences that may favor the survival of altitudinal races in their respective habitats.

Dr. George H. Ward, of Knox College, Galesburg, Illinois, is continuing studies on the *Artemisia tridentata* complex using materials that he assembled some years ago in the Carnegie Institution garden while a graduate student at Stanford University.

<sup>1</sup> Retired June 30, 1956.

His current studies, as a Fellow of the National Science Foundation, include work in cytology, comparative anatomy, and genetics.

Dr. Harlan Lewis, of the Department of Botany, University of California, Los Angeles, was engaged in field and cytological studies on the genus *Clarkia* during a large part of the summer of 1955 at the Mather and Timberline transplant stations, and during the fall of 1955 in the laboratory at Stanford. Dr. Lewis was on a Guggenheim fellowship during a sabbatical year.

Dr. Ursula Brodführer, of the Earhart Laboratory, California Institute of Technology, is utilizing the facilities at the Timberline transplant station to explore the effect of ultraviolet radiation on the growth of *Arabidopsis* and *Mimulus* in connection with studies in the Earhart Laboratory under controlled conditions.

Two members of the staff, Dr. C. Stacy French and Mr. Harold W. Milner, participated in the organization of discussions to evaluate the present status of large-scale algal culture and to review developments in the field since the publication of *Algal Culture: From Laboratory to Pilot Plant* (Carnegie Inst. Wash. Pub. 600). This discussion meeting was a part of the Technical Session held at the University of Arizona October 31 and November 1, 1955, in connection with the World Symposium on Applied Solar Energy arranged by the Stanford Research Institute.

Dr. E. R. Hardwick, of the Stanford University Chemistry Department, and Mr. R. Paul Elliott, of the U. S. Food and Drug Administration, both used the Department's facilities for recording fluorescence spectra; and space in the greenhouse has been used by Dr. Hubert S. Loring, of the Stanford Chemistry Department, for the cultivation of tobacco for virus studies.

Dr. John B. Wolff, of the Smithsonian Institution, and Dr. Arnulf R. Persson, of Oslo, who is temporarily at the University



of California at Davis, both spent about a week with Dr. Smith to become acquainted

with his methods for the analysis of plant pigments.

### EXPERIMENTAL TAXONOMY

Progress in basic investigations described in earlier Year Books, and completion of the manuscript of volume IV of the Institution's monographic series "Experimental Studies on the Nature of Species," have occupied the attention of the Experimental Taxonomy staff during the current year. The studies on *Poa* continue along several lines, and although these investigations have reached an advanced stage, several years will be required for the completion of the grass-breeding program. Further progress has been made in studies on the biology of *Achillea* and *Mimulus*, and in the development of apparatus for quantitative physiological studies on ecologically contrasting ecotypes. Dr. C. S. Venkatesh, of the University of Delhi, India, working on a Smith-Mundt Fulbright fellowship, completed an investigation on the cytogenetics of a group of tarweeds which throws new light on relationships between and within species in a typical group of the sunflower family.

The monograph mentioned in the preceding paragraph deals with the genetic structure of ecological races of *Potentilla glandulosa* and includes an extensive review of literature on the inheritance of complex gene systems that appear to be related to those found in ecologic races of widespread species complexes. Some of the principles developed in this volume were outlined in Year Book No. 47 (1947-1948), pages 106-110.

#### CONFERENCE HONORING DR. VANNEVAR BUSH

The conference on genes, mechanisms, and expression of character, mentioned earlier, in honor of Dr. Bush was attended by the following participants, in addition to the members of the staff: Dr. Irving M. Bailey, Harvard University; Dr. George W. Beadle, California Institute of Tech-

nology; Dr. Roy E. Clausen, University of California, Berkeley; Dr. Harlan Lewis, University of California, Los Angeles; Dr. Charles E. Olmsted, University of Chicago; Dr. C. S. Venkatesh, University of Delhi, India; Dr. Frits W. Went, California Institute of Technology; Dr. Mogens Westergaard, University of Copenhagen; and Dr. Ira L. Wiggins, Stanford University. The objective of the conference was to survey the range of viewpoints, or points of entry, in studies of basic biological questions that are of key importance for an understanding both of the interrelations between plants and their environment, and of mechanisms of evolution.

Viewpoints stemming from those of biochemistry, physiology, anatomy, genetics, taxonomy, and ecology were examined, and current progress in the integration of these fields was evaluated. The extent of existing gaps in knowledge, and modes of approach in bridging the gaps, became the central theme in the discussions. The conferees reviewed the direction of current and future studies in the Department that are designed to further basic advances in the most effective possible way.

Following the conference, a dinner honoring Dr. Bush at Villa Montalvo, Saratoga, was attended by the participants and their wives.

#### STUDIES IN POA

JENS CLAUSEN, WILLIAM M. HIESEY, AND  
MALCOLM A. NOBS

The regional screening tests on parental and hybrid apomictic lines of *Poa* begun last year under the direction of Dr. A. A. Hanson of the U. S. Agricultural Research Service with the co-operation of investigators at state experiment stations, as outlined in the last Year Book (No. 54, p. 172), are now beginning to yield data. Studies of plantings grown in replicated test plots



at Franklinton, Louisiana; Blacksburg, Virginia; Lexington, Kentucky; Lafayette, Indiana; State College, Pennsylvania; Columbia, Missouri; Corvallis, Oregon; and Halls Flat, Lassen County, California were made by members of the staff. At the eastern and midwestern stations Dr. Hanson collaborated with the staff in the comparative surveys.

The data thus far available pertain primarily to the initial establishment of the lines and their survival over one winter in the various areas concerned. It appears that many of the new hybrid lines may have a greater range of tolerance to widely different climates of the United States than was originally anticipated. A combination between *Poa ampla* and *P. compressa*, for example, promises good survival and vigorous growth in Virginia, Louisiana, and Kentucky. Hybrid derivatives from *P. ampla*  $\times$  *P. alpigena*, *P. ampla*  $\times$  *P. pratensis*, and *P. scabrella*  $\times$  *P. pratensis* were, for the most part, successfully established in most of the environments, and it is possible that at some of the stations some may be sufficiently well suited to serve agronomic uses. On the other hand, combinations between *P. scabrella* and *P. ampla*, and certain lines from *P. scabrella*  $\times$  *P. pratensis*, were, as expected, poor under the severe winter conditions of the eastern and midwestern climates. Consistent general patterns of response thus appear to emerge in certain hybrid combinations. Lines from the same parentage may differ sufficiently, however, for one line to be favored strongly at one station, and a sister line at another. Such differences between individual lines are of great importance in agronomic selection.

At Purdue University and The Pennsylvania State University most of the parental and hybrid lines were subjected to turf trials in competition with current local commercial strains. A strain of *Poa pratensis* originally from Newport, Oregon, made a favorable showing as a turf grass in these tests. Extensive studies at the U. S. Soil Conservation Service plantings along the

west coast, at the Institution's altitudinal transplant stations, and at European stations have shown this strain to have a wide climatic tolerance (cf. Year Book No. 54, p. 173).

Another major development in *Poa* is the establishment, for the first time, of stabilized apomictic lines in the interspecific combination *Poa ampla*  $\times$  *P. arida*. Among early interspecific crosses made in 1944, an  $F_1$  derivative between *P. arida* from North Platte, Nebraska, and *P. ampla* from Albion, Washington, proved to be outstanding in vigor. This hybrid was found to be approximately 12 per cent apomictic, a percentage too low to assure its maintenance as a stable apomict. The responses of cloned transplants of the  $F_1$  grown at the altitudinal transplant stations, Stanford, Mather, and Timberline, as well as at the Soil Conservation Service Nursery at Pullman, Washington, indicated that this interspecific combination might be highly desirable for use in cooler mountain climates of California and in areas such as the Palouse Prairie and the Great Basin. During the spring of 1951 additional crosses of this combination were made (see Year Book No. 51, 1951-1952, p. 115). These yielded all together 77  $F_1$  hybrids, all of which, like the original 1944 combination, were of good vigor and high fertility.

During the spring of 1955 progeny of 12 of the 77  $F_1$  plants were grown at Pullman in spaced plantings, and the remaining 65 at Stanford. Among the 77 progenies, 49 segregate freely and are sexual; 18 are less than 30 per cent apomictic; 10 appear to be sufficiently apomictic to warrant further testing, 7 being more than 50 per cent apomictic. Three of these 10 appear to be of special promise. One, although somewhat less than 50 per cent apomictic, surpasses the original 1944 hybrid in vigor and is among the strongest of the hybrids at the mountain stations. It is believed that its extreme vigor and broad range of tolerance may compensate for the relatively low percentage of apomixis when it is planted

in dense stands, as under range conditions. The other two hybrids, although not so vigorous, are 70 per cent apomictic, a degree of uniformity sufficient to assure the stability of a reasonably vigorous line.

An interesting aspect of the *arida-ampla* combinations is the rich array of vigorous recombination types that occurs in segregating  $F_2$  progenies. This is especially evident in the plantings at the Soil Conservation Service Nursery at Pullman. It is conceivable that some of the recombinations may themselves be apomictic; progeny from 27 individuals will be space-planted next year at Pullman by the Soil Conservation Service, and it is hoped that some may prove to be constant apomicts.

During the current season it has been possible for the first time to compare in uniform environments a series of 15 apomictic hybrids all derived from a single sexual  $F_1$  hybrid, but selected at widely different places. The  $F_1$  was a cross between *Poa ampla* originally from Kahlotus, Washington, and *P. pratensis* from the Athabasca region of Canada. Eight promising apomicts were selected from seedling progeny grown at Stanford, five from the Soil Conservation Service Nursery at Pullman, and two at Edinburgh, Scotland, by Drs. J. W. Gregor and Patricia Watson. This series of apomicts has been growing for one year at the Stanford, Mather, and Pullman stations and shows a wide diversity in response patterns. Certain of the strains selected at Stanford for their vigor grow poorly at Pullman, and vice versa, but others are tolerant at both these stations.

The two strains selected at Edinburgh appeared to be uniform and apomictic there. When brought to the Pacific coastal area, one proved to be sexual and the other to be truly apomictic. The apomict is a vigorous strain that thrives especially well at Pullman and Mather, but also grows successfully at Stanford. It is now evident that the range of selection possible among the segregating progeny of a single  $F_1$  hybrid grown in different environments is

extremely wide. This fact is of basic importance both in interpreting the evolution of the complex genus *Poa*, and in the development of forage grasses for agricultural uses.

New cloned transplants of *Poa* representing all key apomictic hybrid lines and their parents were established last year at the Stanford, Mather, and Timberline stations. Their responses are being studied in detail and furnish a basis for evaluating data from the much larger and more diverse field plantings maintained by co-operating federal and state agencies. Dr. Thomas R. Pray, of the University of Southern California, has been working during the past three summers at the mountain stations and has given special attention to the *Poa* plantings.

Cytological studies in *Poa*, including chromosome counts of parental and hybrid lines as reviewed in the last Year Book (No. 54, pp. 175-177) have been largely completed. Miss Lois M. Cox, who worked on the cytology of *Poa* for a year and a half, has taken a position at the University of California at Davis.

Mr. Paul L. Pfahler, graduate student in the Department of Agronomy at Purdue University, worked with the Department during the summer of 1955, reviewing the work in *Poa*.

The Ferry-Morse Seed Company has grown for the Department approximately 180 pounds of seed of an apomictic strain of *Poa scabrella*  $\times$  *P. ampla* raised near San Juan, California. The seed is being distributed to the Soil Conservation Service and other agencies for testing in arid foothill areas having winter rainfall, where this strain appears to have potential value as a range grass.

#### PLANTINGS OF *ACHILLEA* AND *MIMULUS*

Extensive cultures of parental and hybrid populations of members of the *Achillea millefolium* complex and of *Mimulus* at Stanford and at the mountain transplant stations are being continued and studied,

as described in Year Book No. 54, pages 181-183. Investigations in these groups are concerned with cytogenetic relations between species, the genetic composition of natural races, and the responses of segregating  $F_2$  offspring in contrasting environments.

#### CYTOGENETIC RELATIONSHIPS IN SECTION CENTROMADIA OF HEMIZONIA

CHAKRAUARTI S. VENKATESH

During the period between September 1955 and June 1956, while Venkatesh was working on a Smith-Mundt Fulbright fellowship, a cytogenetic study was completed in a unique group of tarweeds. Section Centromadia of the large genus *Hemizonia* consists of four fairly well defined species, two of which, in turn, are composed of recognizable subspecies that occupy distinct climatic zones. Subspecies of *Hemizonia pungens* include *maritima* of the coastal region, *interior* of the central valley of California, and *laevis* of interior southern California. All three have  $n=9$  pairs of chromosomes. *Hemizonia parryi* is similarly composed of four subspecies that tend to parallel those of *pungens* in ecological distribution. The subspecies of *H. parryi*, however, differ in chromosome number, two having  $n=12$  pairs of chromosomes, and the other two  $n=11$  pairs. A third species of section Centromadia, *H. fitchii*, consists of a single subspecies having  $n=13$  chromosomes.

During the years 1932 to 1934 systematic crossings were made among the three species mentioned above, including various combinations of the different subspecies (see Year Book No. 33, 1933-1934, pp. 173-177). Cytological fixations of root-tip and bud material made at that time were utilized in the current study of chromosome numbers and meiosis in the parent species, and in fifteen intra- and interspecific hybrids. These investigations show clearly (1) a high degree of regularity in pairing between chromosomes within the same species, irrespective of whether the sub-

species have the same or a different number of chromosomes; such a regularity of pairing is associated with good seed fertility of the intraspecific hybrids; (2) various degrees of irregularity of pairing in hybrids between different species, with its associated low seed fertility; and (3) a partial homology between the chromosomes of all three species, indicating a basically similar genetic structure of their genomes.

The occurrence of chromatin bridges along with bridge fragments, multivalent pairing, and heteromorphic bivalents between certain hybrids suggests that inversions as well as segmental translocations have played an important role in the process of speciation in this section.

#### PHYSIOLOGY OF CLIMATIC RACES

HAROLD W. MILNER AND WILLIAM M. HIESEY

The desirability of undertaking a study of the comparative physiology of climatic races of plants was discussed in Year Book No. 52, page 176, and the broad outline of the program was stated in Year Book No. 54, page 180. The key to the attack on this problem is the development of a reliable and accurate apparatus for the quantitative measurement of photosynthesis and respiration of intact higher plants under exactly controlled environmental conditions. Such an apparatus is necessarily complex, and requires precise co-ordination of its many working parts. Though its basic principles are simple, a patient step-by-step process of elimination of defects in its operation has been necessary and time-consuming.

A number of troublesome mechanical and electrical peculiarities of the apparatus have been identified and eliminated this year. Improvements in the electronic measuring circuits were made by Mr. John F. Hansen. The response of the carbon dioxide analyzer to changes in concentration is now more linear, has a higher signal-to-noise ratio, and shows improved sensitivity. A change of one part in four million of carbon dioxide can be detected, and the



instrumental response is nearly linear over its full range.

Some of the component parts of the carbon dioxide analyzer were redesigned and rebuilt to improve its mechanical operation. A calibration curve has been made by use of various gas mixtures. Mixtures of air and nitrogen were used for carbon dioxide content less than 300 parts per million ("normal" air concentration), and mixtures of air with added carbon dioxide for higher concentrations. The carbon dioxide content of each mixture, determined by chemical analysis, was then plotted against the cell resistance shown by the analyzer. The working range of the analyzer extends from zero to 600 parts per million of carbon dioxide. By choice of an appropriate concentration of the standard alkali solution used to absorb the carbon dioxide, the range of the analyzer can be extended as desired, but with a proportional loss in sensitivity.

The drifts in apparent carbon dioxide content of the system referred to in Year Book No. 53, page 180, were traced to diffusion or convection transfer of carbon dioxide from the injector unit to the main system. Solenoid valves have been installed at the points of entry of both carbon dioxide and oxygen to the system, and also at the return point of the absorber by-pass. In effect, the injector and absorber units are isolated from the system except during the moments of actual injection or absorption of carbon dioxide or oxygen.

For a brief description of the principles of operation of the apparatus, two greatly simplified and highly schematic diagrams may be helpful. Figure 1 shows the paths of gas movement in the apparatus. The main path (circles) includes the air pump, plant chamber, and dehumidifier. The pump circulates 30 liters of air per minute in this closed system. The pressure difference between the inlet and outlet sides of the air pump is about 1 cm of mercury. This pressure, with a valve to control the flow, is used to send a continuous 150 cc/min sample of the main air stream

through the oxygen analyzer. The carbon dioxide analyzer by means of its own pump (not shown) takes a continuous 300 cc/min sample of the main air stream. Since both analyzers respond to the mass of the constituent analyzed, the indicated values will vary with the pressure at which a given sample is analyzed. For this reason, the return tubes from the analyzers enter the main gas stream at the point where the internal pressure is equal to the external atmospheric pressure.

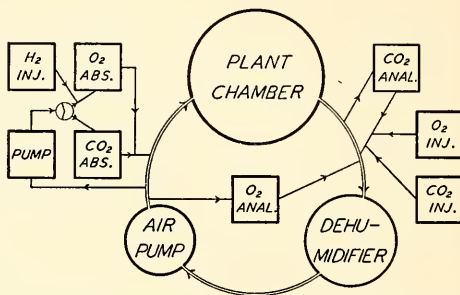


FIG. 1. Diagram of gas flow. Double line, half-inch tubing; single lines, quarter-inch tubing.

Oxygen and carbon dioxide as needed to maintain a constant gas composition of the main air stream are injected into the return tube from the carbon dioxide analyzer.

The absorber pump by-passes 2000 cc/min of the main air stream through the carbon dioxide or oxygen absorber. A three-way solenoid valve directs this 2000 cc/min through one absorber or the other as required.

The carbon dioxide absorber is a helical coil made of 900 cm of 1.5-cm Pyrex tubing. The coil contains 5-normal sodium hydroxide solution to absorb all the carbon dioxide from the air pumped through it. In order to absorb oxygen without introducing into the air stream any chemical vapors harmful to plants, a catalytic process is used. Just before the air reaches the oxygen analyzer, a known quantity of hydrogen is metered into it. The "absorber" is a platinum catalyst on which the



hydrogen combines with its stoichiometric equivalent of oxygen to form water. A solenoid valve (not shown) prevents entrance of hydrogen into the system except when the injector is operating.

Figure 2 shows in skeleton form just those electronic control circuits used in regulating and recording the composition of the gas in the plant chamber. Full lines represent operating signals, broken lines signals to the recorders. The many elec-

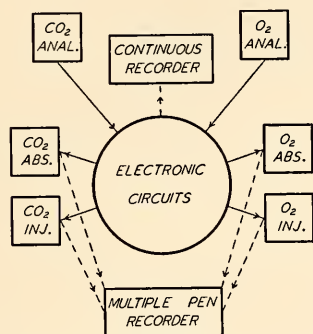


FIG. 2. Circuits for regulating and recording content of carbon dioxide and oxygen.

trical circuits serving for mechanical operation of the apparatus are omitted from the diagram. The analyzers for oxygen and carbon dioxide act only as monitors to detect changes in the content of these two gases in the circulating air stream. If, as a result of the vital activity of the plant, either gas departs from the pre-set norm, the corresponding analyzer will detect the departure and indicate its direction. Immediately the electronic circuits will activate the appropriate injector or absorber to restore the concentration balance of the gaseous constituent which has varied. Simultaneously with the starting or stopping of an injector or absorber, the event is signaled to the multiple pen recorder. From the recorded on-off time of each injector and absorber the quantity of carbon dioxide or oxygen which has been added to or removed from the system can be calculated. Hence, the amount of photosynthesis or respiration accomplished by the

experimental plant may be determined. The continuous recorder shown in the diagram is a recording potentiometer that may be used to keep a continuous record of the concentration of either carbon dioxide or oxygen.

In order to prevent overloading of the system with either carbon dioxide or oxygen, each of the injectors is equipped with a mechanical time-delay device (not shown) which prevents the injection of another portion of gas until the previously injected portion has had time to become thoroughly mixed with the air in the plant chamber.

Latest tests of the improved apparatus indicate satisfactory performance under manual control. Only one hurdle remains before the commencement of measurements using plants. On a long blank run with automatic control the apparatus will have to maintain unchanged the initial concentrations of carbon dioxide and oxygen without the addition or removal of either gas except for replacement of carbon dioxide absorbed in the analyzer.

## PHYSIOLOGICAL STUDIES IN *MIMULUS*

F. J. F. FISHER

In previous reports attention has been drawn to the interesting possibilities provided by members of the genus *Mimulus* for physiological studies of ecological races. Special attention has been given to the two closely related species *M. lewisii* and *M. cardinalis*, which have been shown to behave as ecotypes of a single complex. Problems concerning the seasonal periodicity and transplant responses of these two species have been outlined previously (Year Book No. 54, pp. 181-182).

Further experimental studies are now being carried out with the aim of uncovering in finer detail some of the physiological mechanisms concerned in the adaptation of these species to very different environments. Studies of germination and the growth of seedlings under controlled temperature conditions in small illuminated

cabinets will be followed by direct measurements of respiration and photosynthesis using Warburg manometers as well as the new apparatus being completed in this laboratory. It is also planned to make an assay of the auxin activity of the two species in co-operation with Dr. Winslow Briggs at the Department of Biology, Stanford University.

In the study of seedling growth and development of *Mimulus*, a new technique is being utilized. In order to provide more uniform conditions than is possible in soil cultures, seeds have been germinated on sterile agar in flasks and provided with a complete mineral nutrient supply. Subsequent series of experiments can thus be carried out under reproducible conditions. The sterile culture technique is also likely to prove of value in the proposed studies of respiration and photosynthesis, where metabolic activity of contaminating organisms can be an uncontrollable source of error.

Initial results of these growth studies are in agreement with general observations previously made in the experimental garden and under natural conditions. Germination of both species almost stops at 10° C but takes place rapidly at temperatures of 20° to 30°. The response of the high-montane species *lewisii*, studied with material originating from an altitude of 7000 feet, perhaps reflects a capacity to make use of the very short growing season of its natural habitat. This species not only germinates several days ahead of *cardinalis* from near sea level, but also continues to grow more rapidly at each of the temperatures examined. Thus at the optimum temperature for the montane species, 20° C, the lowland species grows very slowly and has barely unfolded its seedling leaves when the montane species has already formed three fully developed pairs of leaves. At higher temperatures the montane species continues to lead, but with a much reduced margin. The optimum for the lowland species is about 25° C.

Earlier experiments with other species at the Earhart Laboratory have shown that

night temperature by itself is an important ecological factor. In the current experiments, day and night temperatures have been the same. It is planned to carry out further studies of these two *Mimulus* species using different settings of day and night temperatures.

In their natural habitats, *lewisii* and *cardinalis* may grow in soils at very different temperatures. Though both species occupy saturated soils adjacent to running water, in lowland habitats the temperature of this water may be 20° C or more and the soil surrounding the roots correspondingly warm. In montane habitats, however, water often flows directly from near-by melting snow and may be only a few degrees above freezing point. Temperature is known to influence the rate of movement of materials in the conducting systems of plants, and it seems probable that differences in soil temperature may play an important role in fitting these species of *Mimulus* to their respective habitats. In order to examine this possibility, experiments are being planned to vary the temperature of the agar medium separately from the air temperature around the leaves.

## THE BIOLOGY OF SAGEBRUSH

GEORGE H. WARD

A grant from the National Science Foundation has made it possible to continue, during summer periods, work on the biology of the *Artemisia tridentata* complex. Previous studies on this group were discussed in Year Books No. 49, page 109, and No. 50, pages 112-120, and in a paper published in *Contributions from the Dudley Herbarium*, volume 4, pages 155-205, 1953. The principal goal of the present study is to determine, as far as possible, the morphologic, ecologic, and geographic limits of the various genetic races of sagebrush. Surprisingly little is known concerning the basic biological structure of this group of plants that occupies vast areas of arid western lands.

Field work, herbarium studies, and cytological information brought together in

earlier years have contributed to a tentative taxonomic treatment outlining the species and major subspecies of this confusing and unique group. The current studies are being carried on both in the field and in experimental gardens. Between 1949 and 1951 living plants of different species and many races from a number of western states were transplanted to the garden at Stanford. These plants, now well established, are in prime condition for further study and experimentation. It is anticipated that the information that can be gained, when reviewed on the background of field studies, will shed much light on the biological structure of the various species, especially *Artemisia tridentata* itself.

The present work at Stanford includes the following:

1. A comparative study of growth, blooming period, and morphology of garden-grown plants. Preliminary observations indicate that length of growth period in the various ecological races is genetically controlled. Time of bloom depends on the length of the summer growth period, and not on external factors except as these determine the beginning, or modify the length, of the growth period. Collections made in the field from the original trans-

plants are now preserved in the Dudley Herbarium at Stanford University and furnish information useful for comparing garden-grown with field-grown plants.

2. The collection of bud fixations for the study of chromosome pairing during meiosis. Fixations made in the field have not been successful, but plants grown in the garden may be checked daily to ensure obtaining the proper stages. Knowledge of the behavior of chromosome pairing in diploids, in the various kinds of polyploids, and in supposed hybrids is important for interpreting the numerous kinds of variation observed in the field.

3. Attempts at hybridization. Progeny from successful crosses would furnish authentic hybrid material for study, and the degree of crossability between different combinations would furnish information of critical importance regarding degrees of relationship. Crossings can be made only between forms whose blooming periods overlap when grown at Stanford.

On the background of information gained from such garden studies, further field exploration, especially in the Rocky Mountains, where the ecologic and morphological variation within the *Artemisia tridentata* complex is greatest, can be undertaken with enhanced effectiveness.

## BIOCHEMICAL INVESTIGATIONS

### ON THE PREPARATION, PURIFICATION, AND NATURE OF THE PROTOCHLOROPHYLL HOLOCHROME

JAMES H. C. SMITH, DONALD W. KUPKE,  
AND ARTHUR T. GIESE

Experiments in the laboratories of the Department during the past several years have furnished quantitative evidence that protochlorophyll, a pigment contained in dark-grown leaves, is transformed exclusively to chlorophyll *a* when these leaves are illuminated under the proper conditions. These experiments have also shown that protochlorophyll can be extracted from the leaf with glycerine or with aqueous buffer solutions in a form that still retains a large proportion of its transformability.

The protochlorophyll in the leaf and in these extracts is combined with a carrier; we have given this combination the name protochlorophyll holochrome. We have undertaken to determine in what manner and with what kind of carrier the protochlorophyll must be combined in order to be phototransformed to chlorophyll.

The most definitive information regarding the character of the protochlorophyll holochrome can be obtained by determining the properties of the isolated pigment complex. A major effort has been made to isolate the holochrome.

Although the protochlorophyll holochrome can be extracted from several plant sources by glycerine in an active form,



i.e., in a form that can be transformed to chlorophyll *a* by light, no method has been found for separating the active material from the glycerine extracts. Recourse has been had, therefore, to extraction of the pigment from dark-grown bean leaves by aqueous buffer solutions, since this kind of extract is the only one found so far from which active fractions can be separated. The activity of the material is measured spectrophotometrically by observing the change caused by illumination from the characteristic absorption spectrum of protochlorophyll to that of chlorophyll *a*; see figure 3.

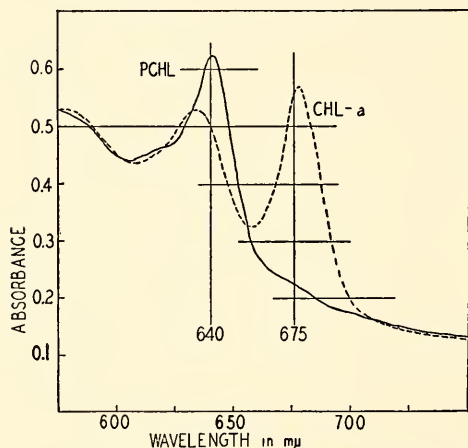


FIG. 3. Absorption spectra of the protochlorophyll holochrome and of the chlorophyll-*a* holochrome derived therefrom by the action of light. Solvent: 0.1 M glycine-0.05 M potassium hydroxide, pH 9.5. The general upward trend of the absorption toward shorter wavelengths is attributable to nonselective scattering. The position and height of the second chlorophyll-*a* band, 633 mμ, are undoubtedly changed somewhat by the absorption of untransformed protochlorophyll.

In order to obtain active extracts of the pigment complex, the dark-grown bean leaves are ground with mortar and pestle in a suitable buffer solution at about 5° C in the dark. The extract is freed of fibers by straining through a linen cloth and then centrifuging to remove coarse debris. The supernatant solution is centrifuged further

for 1 to 3 hours at 10,000g to clarify the extract and to remove all components sedimenting faster than the holochrome. Extracts obtained by this routine procedure are either stored in the cold and dark for future use or treated further by specialized methods.

Several kinds of buffer solution have been used successfully for extracting the active pigment complex: phosphate, arsenate, carbonate-bicarbonate, glycine-potassium hydroxide, and veronal. Sucrose (0.88 M) in the buffer solutions is beneficial, especially with carbonate buffer. For obtaining good extraction and retention of activity, the pH of the buffer solution should be between 6.5 and 10. Below pH 6 the holochrome is insoluble and therefore is not extracted, and above pH 10.35 the pigment is extracted but inactivated.

The stability of the extracts is affected by the kind of buffer solution used, and is best in the glycine-potassium hydroxide and the carbonate-sucrose solutions. It is also affected by the concentration of the buffers, being much better at low ionic strengths. Since low salt concentrations are desirable, a compromise must be made between low salt concentration and adequate buffering capacity. Ionic strengths of about 0.05 were most often used. The stability is also affected by temperature, so the extracts were made and stored at temperatures near 0° C.

Extracts made by the routine procedure and dialyzed against buffer solution lose but a small fraction of their capacity for pigment transformation. This demonstrates that no diffusible cofactors are necessary for the phototransformation. Since the protochlorophyll does not diffuse out of the dialysis sac, it must be attached to a relatively large molecule or particle. Dialysis achieves some purification of the protochlorophyll, as is shown by the fact that a considerable quantity of yellow material diffuses from the sac and a precipitate forms which has little activity and is removed by centrifugation.

The extract is next dialyzed against dis-



tilled water. This frees the protochlorophyll solution from buffer and readies it for freeze-drying. Removal of the water by freeze-drying produces a fluffy, slightly yellow, active residue.

The characteristic absorption spectra of the dialyzed glycine-potassium hydroxide buffer extract of the protochlorophyll complex and of the chlorophyll-*a* complex obtained therefrom by the action of light are shown in figure 3. This solution when subjected to ultracentrifugal analysis yielded valuable information concerning the natural pigment. Several analyses by the Spinco Model E ultracentrifuge showed the presence of two distinct boundaries: one, slow-moving, with sedi-

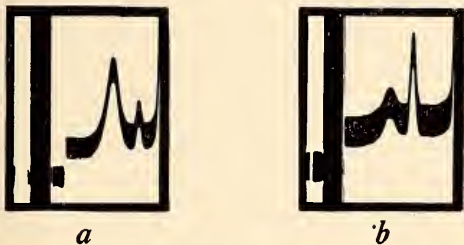


FIG. 4. Sedimentation diagrams showing the degree of purification of the protochlorophyll holochrome achieved by ammonium sulfate fractionation: (a) pattern before fractionation; (b) pattern after fractionation. Sedimentation is toward the right. In each photograph, the pattern demonstrates the presence of two components with sedimentation coefficients in Svedberg units of 3 to 4, left peak, and 16 to 17, right peak. Centrifugation conditions: Spinco Model E ultracentrifuge, synthetic boundary cell, 50,740 rpm; solvent medium, 0.1 M glycine-0.05 M potassium hydroxide, pH 9.5; both photographs taken 17 minutes after attaining operating speed.

mentation coefficients ( $s_{20}$ ) of 3 to 4 Svedberg units ( $S = 1 \times 10^{-13}$  sec); the other, faster-moving, with sedimentation coefficients of 16 to 17 S (see fig. 4a). The sedimentation coefficients have been corrected to 20° C in pure water. By centrifuging the dialyzed solution in a Spinco Model L preparative centrifuge at an average gravity of 91,000 for 3 hours a pellet was obtained which when dissolved showed a greater

absorption corresponding to protochlorophyll than did the supernatant solution, and by ultracentrifugal analysis a much greater proportion of the faster-moving (16 to 17 S) component. This proved that the protochlorophyll holochrome was associated with the faster-moving component.

Since the active protochlorophyll holochrome could be separated from extracts by centrifugation, it seemed probable that it could be purified by repeated high-speed centrifugation. This proved to be impossible, however, because the pellets did not dissolve appreciably after a second such centrifugation, and the activity of the dissolved portion was very low; denaturation of the protein during the high-pressure packing appeared to be involved. Other methods of purification were therefore tried.

One method examined was that of selective adsorption. Both columnar adsorption and batch adsorption were investigated. Of the adsorbents tested, calcium phosphate showed greatest promise of usefulness; of the solvents tested, ammonium phosphate buffers of various hydrogen ion concentrations seemed to be most effective. In these experiments, the pigment complex was adsorbed on calcium phosphate from ammonium phosphate buffer at pH about 6.5 and eluted from the adsorbent by ammonium phosphate at pH about 9.8. Not all the protochlorophyll was adsorbed at the lower pH. This indicates that more than one kind of protochlorophyll holochrome is present. Whether these holochromes differ in respect to carrier, to association of pigment and carrier, to degree of aggregation, or to other characteristics can only be surmised at present. Most of the adsorbed pigment was eluted, and the eluted pigment was transformable by light. Some purification was obtained, but whether complete purification can be accomplished by these methods is yet to be determined.

The electrophoretic behavior of the extracted materials suggests that the protochlorophyll holochrome could perhaps be

isolated and purified by electromigration methods. Zone electrophoresis of the crude mixtures on paper strips, on columns of cellulose powder, or by use of the Kolin method, which depends on the differential electromigration of the ions to be separated in a concentrated sucrose medium, has separated the sample into several fractions. At pH 6.8 and above, the active holochrome migrated to the positive electrode.

Electrophoresis by the Kolin method brought about separation into three distinct bands: a leading yellow-fluorescing band, an intermediate band exhibiting Tyndall scattering and the characteristic red fluorescence of the holochrome, and a trailing band with strong Tyndall effect but with no fluorescence. When a solution of a pellet obtained by high-speed centrifugation was used in the Kolin procedure, the leading yellow-fluorescing band was nearly absent. The bands were always sharply separated from one another. If the method could be developed to handle larger quantities of material, it might prove to be excellent for purification of the holochrome. As yet, none of the electrophoretic methods have produced purified materials in quantity sufficient for chemical work.

In addition to centrifugation, adsorption, and electrophoretic techniques for the separation and purification of the protochlorophyll holochrome, salt precipitation methods have been tested. Basic lead acetate in low concentrations in alkaline solution precipitated the holochrome. When the precipitate was exposed to light, the protochlorophyll was partially transformed to chlorophyll, as indicated by the absorption spectrum of an ethanol extract. Zinc sulfate (0.026 M) also precipitated the protochlorophyll complex from alkaline solution, and the precipitate readily dissolved in Versene solution, pH 9.25. Absorption measurements on this solution demonstrated the presence of intact protochlorophyll holochrome with an absorption maximum at 635 m $\mu$ , which was transformed by light to chlorophyll-*a* holochrome with an absorption maximum at 675 m $\mu$ . Fur-

ther attempts to recover the holochrome from the Versene solution have as yet been unsuccessful. Fractionation of the holochrome by stepwise precipitation with ammonium sulfate has given by far the most encouraging results.

The ammonium sulfate precipitation was carried out in the dark at  $-5^{\circ}$  C. Ammonium sulfate at 30 per cent saturation precipitated a small fraction of the active component contaminated with a large quantity of foreign material; at 36 to 40 per cent saturation it precipitated the bulk of the active material admixed with a relatively much smaller proportion of impurities; and at 50 to 67 per cent saturation it precipitated the main portion of the slow-moving (3 to 4 S) component. Precipitates of the active material, obtained from solutions 36 to 40 per cent saturated with ammonium sulfate, were dialyzed against 0.1 M glycine-0.05 M potassium hydroxide buffer at pH 9.6 until the solution was very clear. The holochrome was then reprecipitated with ammonium sulfate, and the fraction at 34 to 40 per cent saturation was collected. This precipitate, after being dialyzed, gave the ultracentrifuge diagram shown in figure 4*b*. When figure 4*b* is compared with the typical diagram of a sample not fractionated by ammonium sulfate precipitation, figure 4*a*, it is clear that the relative proportions of the two components have been reversed, and that a considerable increase of the protochlorophyll holochrome, which is correlated with the leading boundary, has been achieved by the ammonium sulfate fractionation. A rough estimate made from the ultracentrifuge diagrams indicates that the active protochlorophyll component comprises from 5 to 10 per cent of the two components in the unfractionated sample (fig. 4*a*), whereas it comprises about 75 per cent in the fractionated sample (fig. 4*b*). Experiments are now in progress to further reduce the amount of coprecipitation of the slower-moving component by ammonium sulfate.

Characterization of the holochrome par-

ticles by ultracentrifugation is yet in its early stages, since greater purity is necessary before a detailed description of their sedimentation behavior can be undertaken. Nevertheless, pertinent information about the particle has been obtained by this technique. During ultracentrifugation, the moving boundary of the holochrome particles has always remained relatively sharp—its spreading has been symmetrical but not extensive. Artificial sharpening of the boundary (a result of pronounced asymmetry in particle shape) has not been observed when the rotor speed was increased. In all experiments, a strictly linear relation has been obtained when the natural logarithm of the distance between the boundary and the axis of rotation has been plotted against time of sedimentation. Finally, the sedimentation coefficient,  $s_{20}$ , obtained from the slope of this line has shown only a slight upward trend with decreasing particle concentration.

These observations suggest that no obvious heterogeneity of particle size exists for the molecular species represented by this boundary; and that these particles are very symmetrical in shape, perhaps nearly spherical, so that when the absolute concentrations can be determined, the  $s_{20}$  at infinite dilution will not be substantially greater than 17 Svedberg units. Comparison of this  $s_{20}$  value with those published for globular proteins places the molecular weight of these particles at about 400,000. In making this comparison, it is assumed that the density of the particles is in the range of densities of simple proteins. This assumption seems justified for the following reasons: convincing evidence exists for the protein character of these particles; neither lipids nor nucleic acids appear to be present, to any great extent, in the most purified preparations; and the sedimentation rates of these particles in solutions of various densities parallel the results obtained with other proteins.

The sedimentation properties of the particles demonstrate beyond doubt that the active protochlorophyll is contained in units

much smaller than such cellular organelles as mitochondria, microsomes, or proto-plastids. Filtration of active particles through Millipore Filters which pass only particles smaller than 0.43 microns in diameter also rules out the presence of larger-sized bodies. The scattering of different wavelengths of light outside the region of selective absorption indicates that the size range of the particles lies close to that associated with Rayleigh scattering. These facts support the ultracentrifuge results, which show that the protochlorophyll holochrome possesses properties of a macromolecule with a molecular weight of approximately 400,000.

Undoubtedly this macromolecule consists largely of protein, for it is precipitated by protein precipitants, for example, heavy metals, ammonium sulfate, and perfluoro-octanoic and trichloroacetic acids. Also, the best preparations obtained so far exhibit an absorption maximum at 276 m $\mu$ , a region in which almost all proteins absorb. Since these preparations lack the absorption maximum at 260 m $\mu$  characteristic of nucleic acid, the pigment complex is probably not a nucleoprotein. Separation of the protochlorophyll holochrome by selective adsorption or precipitation has always demonstrated close correlation between the magnitude of the characteristic fluorescence or absorption of a given fraction and the quantity of precipitate produced by addition of trichloroacetic acid.

Many factors that might affect the transformation of the protochlorophyll to chlorophyll *a* have been examined. Lyophilization does not destroy the activity of the holochrome. When the lyophilized material is dissolved and then illuminated, the protochlorophyll is transformed. Photo-transformation also takes place in the solid material after thorough drying over stick potassium hydroxide, a fact which suggests that this photochemical process is independent of the presence of free water. The transformation can be followed spectrophotometrically in the solid lyophilized material by means of the opal glass tech-



nique originated by Dr. Shibata, for whose collaboration we are grateful. Storage of the lyophilized material in the cold and dark for months has no demonstrable effect on its transforming capacity. The lyophilized material has one property in common with dried leaf material: the pigment is not dissolved by absolute acetone, but is removed by 80 per cent aqueous acetone.

The activity of the holochrome is not destroyed by p-chloromercuric benzoate, an inactivator of sulfhydryl groups; by hydroxylamine, which inhibits photosynthesis; by formaldehyde, which could possibly react with active free amino groups; by thiobarbituric acid, which could react with heavy metals; or by 3-amino triazole, which acts as a weed killer by inhibiting chlorophyll formation. (We are indebted to the American Cyanamide Company for giving us the 3-amino triazole.) The transformation is stopped by the detergent sodium deoxycholate.

A number of biologically important substances were tested, in co-operation with Dr. Bernard L. Strehler, for their effectiveness in promoting the transformation in darkness or inhibiting it in light. The substances tried were diphosphopyridine nucleotide (DPN) and its reduced form (DPNH), triphosphopyridine nucleotide (TPN) and its reduced form (TPNH), adenosinetriphosphate (ADP), ascorbic acid, and cysteine. None of these substances brought about transformation in the dark or inhibited it in the light.

The following anions in concentrations of the order of 0.01 M or higher have shown no effect on the phototransformation: cyanide, arsenate, pyrophosphate, hydrosulfite, carbonate, sulfate, phosphate, chloride, acetate, and citrate. Likewise the cations sodium, potassium, ammonium, magnesium, calcium, zinc, and lead appear to be without effect. The phototransformation was largely blocked in Tris buffer and partly blocked in diethanolamine buffer, each at 0.05 ionic strength.

During the past year, considerable progress has been made toward the isolation,

purification, and characterization of the protochlorophyll holochrome. At the present writing, this holochrome is believed to be a combination of the pigment, protochlorophyll, with a protein whose molecular weight is about 400,000. Preliminary calculations indicate that the molecule contains no more than two protochlorophylls. Purification procedures have never completely removed carotenoid pigments. The definitive characterization of the holochrome still awaits its rigorous purification. The purification of this particle has assumed added significance since a search of the literature has revealed that a protein fraction from green leaves was described a few years ago (Singer *et al.*, 1953) which had the same sedimentation and solubility properties as the holochrome particles we have found in etiolated leaves. No function was noted, however, for the green-leaf protein. Only this year it was reported (Lyttleton, 1956) that this protein fraction appears to occur exclusively in tissue having photosynthesizing activity, and indications are that the carboxylation enzyme for carbon dioxide fixation in leaves (Weissbach *et al.*, 1956; Lyttleton, unpublished) is contained, at least, in that fraction.

#### SPECTROSCOPIC STUDIES ON CHLOROPHYLL FORMATION IN INTACT LEAVES

KAZUO SHIBATA

We have followed the transformation of protochlorophyll *a* into chlorophyll *a* by observing the absorption spectra of intact leaves. The difficulty which had previously prevented satisfactory spectroscopic observations of intact etiolated leaves with so little absorbing material was due to the scattering of light by the other leaf components. This difficulty has been overcome by using opal glass for the complete diffusion of the light, as recently described by Shibata, Benson, and Calvin.

A Beckman DK-2 spectrophotometer was used with opal glass plates. The samples were etiolated pole bean leaves which had been cultured in the dark for



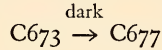
1 to 2 weeks. The original etiolated leaves showed the main bands of protochlorophyll *a* at 650 mμ and at 445 mμ, in agreement with the positions of the bands in the action spectrum of protochlorophyll transformation observed by Koski, French, and Smith (1951). In addition to the bands of protochlorophyll *a* and of carotenoids, there was a band at 636 mμ, particularly in the spectrum of the older (2 weeks) etiolated bean leaves. By the action of light the band of protochlorophyll *a* at 650 mμ was immediately transformed into a newly discovered band at 684 mμ, while the band at 636 mμ was not changed. The etiolated leaves cultured for 7 to 10 days contained much less of the compound that absorbs at 636 mμ than did older leaves. The main studies of the process of the formation of chlorophyll were, therefore, carried out with etiolated leaves cultured for 7 to 10 days.

To simplify the discussion, each compound is designated by its initial letter with the wavelength of its absorption maximum in the red region of the spectrum. Thus, active protochlorophyll *a* is called P650. The first product formed by the action of light has an absorption band at 684 mμ. This new form of chlorophyll is designated C684.

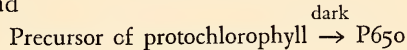
An etiolated leaf was first illuminated (1000 foot-candles white light for 1 minute) and then kept in the dark, and the further changes of the spectrum of the leaf in the dark were followed. Within about 20 minutes in the dark after illumination, the band at 684 mμ disappears and a band at 673 mμ appears. In this process we observe a definite isobestic point, which indicates that the process is a transformation from one single compound to another

<sup>dark</sup>  
(C684 → C673). After about 20 minutes in the dark, different kinds of changes are observed in the spectrum. These further changes appear more clearly after 40 to 60 minutes in the dark, because they are then not obscured by the overlapping spectral effects due to the initial change (C684 →

C673). In this second dark process the band at 673 mμ is gradually shifted to one at 677 mμ, which agrees with the absorption maximum of chlorophyll *a* in mature green leaves. At the same time, the protochlorophyll *a* band reappears at 650 mμ. Therefore, in this 40- to 60-minute period the following two processes occur:



and



These processes take about 1 hour, starting after a lag period of 20 minutes in the dark after the illumination. To make sure that the band which reappears at 650 mμ is due to protochlorophyll, a leaf that had been kept in the dark for 100 minutes following the first illumination was reilluminated. This illumination caused the band at 650 mμ to disappear and simultaneously the band at 684 mμ to reappear. This spectral change is similar to that induced by the first illumination, which is caused by the transformation of protochlorophyll to chlorophyll *a*. Undoubtedly, therefore, the band reappearing at 650 mμ is due to reformed protochlorophyll.

From the experiments in this study it is clear that two intermediates (C684 and C673) appear between protochlorophyll *a* (P650) and chlorophyll *a* (C677) in the natural conversion process. At the stages of conversion in which these intermediate pigments appeared, the leaves were extracted with ether in order to determine the absorption maxima of the intermediates in this solvent. The absorption maxima of both the extracted pigments in the red region of the spectrum lay at 663 mμ, which agrees with the well known absorption maximum of chlorophyll *a* in ether. The identification of the absorption maxima of the intermediates in the Soret region was not successful because of the overlapping absorption by the carotenoids. These results tell us that C684 and C673 are compounds very similar to chlorophyll *a*, or are compounds which can be easily

converted into chlorophyll *a* in the course of extraction. Possibly they are chlorophyll *a* in physical states *in vivo* differing from the chlorophyll *a* (C677) in the final state in mature green leaves. More extensive studies, including chemical experiments, will be needed to clarify the detailed mechanisms of the processes found in these spectroscopic observations on intact leaves.

THE PARTICIPATION OF  $\beta$ -CAROTENE IN  
PHOTOCHEMICAL REDUCTION BY  
CHLOROPLASTS

VICTORIA H. LYNCH AND C. STACY FRENCH

Many attempts have been made to separate leaf extracts or chloroplasts into a pigment fraction and a protein fraction and to recombine the two fractions into a unit with some sort of photochemical activity. Most experiments of this type have given negative results and have not been published. There are, however, two reports from other laboratories in which some reactivation was described although the amount of reactivation was at the lower limit of the detection methods used and the positive effect was somewhat elusive.

We hoped that a more sensitive method for detecting the formation of a chemical reductant by a plant system might lead to more positive and consistent results. Reduction of the dye 2,6-dichlorophenolindophenol has frequently been used to study photochemical reduction by isolated chloroplasts. A rapid sensitive method using a Beckman DK-2 recording spectrophotometer was developed to measure the amount of reduction of the dye. Opal glass plates were used to eliminate the effect of scattering. All our experiments on the readdition of chlorophyll to chlorophyll-free chloroplast protein preparations failed to give detectable photochemical reduction of the dye.

Lyophilized chloroplast preparations have about 90 per cent of their original photochemical activity, and when stored in the cold and dark retain much of this activity for several months. It was found

that these dried chloroplast preparations when extracted briefly with cold petroleum ether lost over 80 per cent of their photochemical activity. When the petroleum ether extract was added back to the extracted chloroplast residue and the petroleum ether evaporated, however, nearly the full photochemical activity was regained. Petroleum ether extraction of the dried chloroplasts removes only about 5 per cent of the chlorophyll but much of the yellow pigments.

The petroleum ether extract was separated into three fractions on a magnesium oxide adsorption column. Two yellow bands as well as the colorless fraction were tested for their ability to reactivate the extracted chloroplasts. Of the three fractions, only one of the yellow bands produced activation when added to the chloroplast residue. This band had an absorption spectrum almost identical with that of  $\beta$ -carotene. Activation was obtained when  $\beta$ -carotene was added to the previously extracted chloroplast residue by evaporation of a petroleum ether solution.

Therefore, it is now possible to remove one of the pigment components of the chloroplast system, thereby causing inactivation, and to add it back or replace it in a simple and easily reproducible way. This finding opens the way to a more direct experimental attack on the role that carotenoids play in photosynthesis.

Since under suitable conditions  $\beta$ -carotene could be successfully removed from the chloroplast system to produce inactivation and then added to give reactivation, the removal and addition of chlorophyll was again attempted using similar techniques. Lyophilized chloroplast fragments were extracted with cold acetone to remove chlorophyll. The acetone-extracted chloroplast residue was photochemically inactive. When the acetone extract was added to the chloroplast residue and the acetone removed by the identical method used to remove petroleum ether in the previous experiments, the resultant chloroplast material was still inactive. Chlorophyll can-

not yet be removed and replaced to yield photochemically active chloroplast material as easily as can  $\beta$ -carotene.

#### ABSORPTION SPECTRUM CHANGES IN CHLORELLA CAUSED BY LIGHT

BERNARD L. STREHLER AND VICTORIA H. LYNCH

A reinvestigation of the light-induced absorption spectrum changes in *Chlorella* was undertaken to compare the absorption changes with the delayed light production of the same material under a variety of conditions. This delayed light production was found to be correlated with the absorption changes in relation to illumination intensity and to time. It was concluded that the energy for light emission is stored in those products formed by a previous light exposure that are detectable by their own change in absorption. The absorption changes at about 480 and 520 m $\mu$  described by Duysens and by Witt were confirmed, and smaller changes at 550 to 600 m $\mu$  similar to those previously investigated by Lundegardh, and attributed by him to cytochromes, were observed. New bands dependent on illumination were found at 645 and 660 m $\mu$ .

The absorption measurements were made with the derivative spectrophotometer modified to record the difference between two beams of the same wavelength. The luminescence measurements were made with a photomultiplier used as a quantum counter that was brought from the University of Chicago. Both direct illumination of the cells during the absorption measurement and illumination in a flowing system were used to permit the choice of a variable illumination period followed by a variable dark period before the absorption measurement.

Whether the absorption increases or decreases at a given wavelength depends on the time of illumination and the following dark period. With direct illumination the changes were in the same direction as found by Duysens and by Witt. In the dark after illumination there was an over-

shoot in the opposite direction when the absorption was measured at 525 m $\mu$ . In the flowing system conditions could be adjusted to measure absorption during the overshoot period by illuminating for 30 seconds and then allowing a 5-second dark period. The absorption changes were measured in this way from 470 to 670 m $\mu$ . A partial denaturation by heating the algae to 51° C for 4 minutes produces a system capable of changing absorption in response to illumination, but the peaks of the difference spectrum, though higher, are much broader. Heating to 60° C for 5 minutes abolishes all changes of absorption by light.

In collaboration with Dr. J. H. C. Smith some experiments were performed on chemiluminescence of chlorophyll in methanol solution, produced by the addition of aldehydes. A shift of the red absorption band from 666 to 645 m $\mu$  was found when aldehyde was added to the chlorophyll solution in the presence of air or of tertiary butyl peroxide. It was found that a number of colored reaction products were formed by the luminescence reaction.

In the chlorophyll and aldehyde systems there are at least two reactions of chlorophyll *a* or its products which can produce light. The first takes place in total darkness and is completed at room temperature in about an hour. The second luminescence reaction occurs when such a reaction mixture, after exhausting itself, is exposed to light; thereupon a bright luminescence, comparable to that occurring in the dark with fresh chlorophyll, again occurs.

#### SELECTIVE SCATTERING OF LIGHT BY PIGMENTED ALGAL CELLS

PAUL LATIMER

In a series of measurements recently completed at the University of Illinois, we observed a strong spectral selectivity in the scattering of light by pigmented algal cells. Sharp scattering maxima were found on the long-wavelength side of absorption bands.

For these measurements, cell suspensions



were illuminated with a monochromatic beam. The intensity of the light scattered at  $90^\circ$  to the incident beam was compared with the intensity of the incident light. Corrections were made for the attenuation of the incident and scattered beams by cells in the suspensions other than the observed scattering cells and for fluorescence from the scattering cells.

The scattered light originates from two sources in the cell: (1) the colorless structures, which scatter with a relatively uniform dependence on wavelength, and (2) the highly pigmented chloroplasts (or grana), which scatter with a strong spectral selectivity. The capacity of a particle, such as a granum, to scatter light should increase with its index of refraction. According to the classical theory of dispersion, the refractive indices of a particle for various wavelengths of light pass through maxima on the long-wavelength side of absorption bands. This is in fact where we found the scattering maxima.

By measuring the wavelength dependence of scattering, we are thus able to distinguish between light scattered by groups of pigment molecules in the cell and that scattered by its colorless structural elements. Other studies of light scattering have provided valuable information about the size and shape of materials such as proteins, synthetic polymers, aerosols, and particles in interstellar space. It appears likely that the theories and techniques which have been successfully used in the study of these substances should lead, when applied to studies of *selective* scattering by living cells, to quantitative information about the size, shape, density of packing, and perhaps arrangement of submicroscopic groups of pigment molecules in the cell. Such information is badly needed in order to correlate our knowledge of the very fine structure of cells, as revealed by the electron microscope, with the spectroscopic data at hand.

An important step that remains before specific information about pigments in their natural state may be obtained is the

correction of experimental scattering curves for reabsorption of nonselectively scattered light within the primary scattering cells themselves. This correction has not yet been determined, since it would require in addition to the data at hand a detailed knowledge of the structure and optical properties of the cell. Though the asymmetry of the observed scattering curves about absorption maxima precludes the possibility that the observed selective scattering is due merely to selective absorption of nonselectively scattered light, it is likely that this factor does affect our results to some extent.

Despite this difficulty, we have been able to obtain some information about the size of the groups of molecules which must be responsible for selective scattering. We compared the light scattered by live cells at  $45^\circ$  with that at  $135^\circ$  from the direction of the incident beam. In *Chlorella*, the results indicated that the selective scattering was due to groups of pigment molecules whose dimensions are appreciably greater than  $500 \text{ \AA}$ . This finding is consistent with what might be expected on the basis of electron micrographs.

At the Department of Plant Biology we have found that light scattered at small angles may be measured with the Beckman DK-2 recording spectrophotometer using suitable baffles and plates of ground or opal glass. Preliminary measurements with this system have revealed a spectral selectivity in the light scattered at approximately  $15^\circ$  to  $30^\circ$  from the incident beam which is similar to that previously found for the light scattered at  $90^\circ$ . Further experiments, including the determination of the degree of polarization of selectively scattered light, are also being undertaken.

#### SPECTRAL MEASUREMENTS OF TRUE ABSORPTION AND REFLECTION OF TRANSLUCENT MATERIALS

KAZUO SHIBATA

In order to determine the true transmission, absorption, or reflection of a light-



scattering sample, it is necessary to measure the light emerging from the sample in all directions. The system commonly used to determine the total light emerging, regardless of its direction, is to measure the brilliance of the inside wall of a white sphere totally enclosing the sample. Numerous and subtle errors are involved in the use of such a sphere, due to the finite size of the necessary openings and to the illumination of the sample by the sphere walls as well as by the incident beam. Various correction factors must be considered, and the use of such integrating spheres often becomes complicated in practice.

We have developed a system, adaptable for use in most commercial recording spectrophotometers, which is capable of giving results at least as reliable as sphere measurements and with far less difficulty. Opal glass plates, which may be used with nearly any existing spectrophotometer, are arranged to diffuse the light in such a way that a representative fraction of the total light from either the front or the back surface of the sample may be measured. This method is not so suitable as the sphere for very thick samples that emit a large part of the light from their sides. But for leaves or for cellular suspensions in relatively thin vessels, the opal glass method gives precise results with ease.

A previously described method for obtaining sharp absorption spectra of scattering materials, developed by Shibata, Benson, and Calvin, used opal plates directly behind the sample and the reference cell. This system is entirely suitable for determining the shapes of the absorbance curves of translucent samples, although a small correction for the effect of multiple reflections between the opal glass and the sample is necessary to obtain the absolute absorbance. But such a measurement is not a quantity from which we can calculate the light absorbed by a sample, since the reflected light comes into the measurement as though it were absorbed rather than reflected.

A means of using opal glass to measure

the absolute diffuse reflectance was developed and used for the redetermination of the reflection coefficients of magnesium oxide in the visible spectrum. By combining the diffuse reflectance measurement with the diffuse absorbance measurement, using opal glass on the incident-light side of the sample, it is possible to measure the absolute absorption of thin, light-scattering samples.

To obtain the precise value of diffuse absorbance, it is necessary to make a correction for the reflected light from the back surface of the sample that re-enters the sample because of reflection by the opal

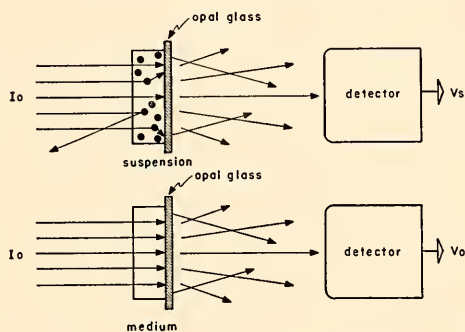


FIG. 5. Measurement of absorbance by parallel incident light

glass. This correction is obtained by means of a gray film of measured diffuse transmittance inserted between the opal glass and the sample.

These methods are illustrated in figures 5 and 6. Figure 5 shows the method of Shibata, Benson, and Calvin for obtaining true absorbance by parallel incident light, including the correction for the multiple reflections mentioned above. The detector output voltages are  $V_s$  for the sample beam and  $V_o$  for the reference beam. The quantity plotted by the recorder is  $\log(V_o/V_s)$ . The other terms used are defined as follows:

$I_0$ , intensity of parallel incident light;  
 $T_s$ , transmittance of the sample;  
 $T_1$ , transmittance of the opal glass;  
 $R_s$ , reflectance of the sample;

$R_1$ , reflectance of the opal glass;  
 $b$ , the fraction of light transmitted by the opal glass that is measured by the detector multiplied by the detector sensitivity.

The sample beam detector output is

$$V_s = bI_0 T_s T_1 (1 + R_1 R_s + R_1^2 R_s^2 + R_1^3 R_s^3 + \dots) \\ = \frac{bI_0 T_s T_1}{1 - R_1 R_s}.$$

The reference beam detector output is

$$V_0 = bI_0 T_1.$$

The apparent absorbance so measured,  $E_{p1} = \log(V_0/V_s)$ , is related to the true absorbance,  $E_p = \log 1/T_s$ , for parallel incident light by the relation  $E_{p1} = E_p + \log(1 - R_1 R_s)$ .

Now, the effect of multiple reflection may be removed by placing a thin gray film of transmittance,  $T_f$ , between the sample and the opal plate and also in front of the reference plate. The apparent absorbance for the sample so measured is:

$$E_{pf1} = E_p + \log(1 - R_1 R_s T_f^2).$$

A value of  $T_f = \text{ca. } 0.2$  is suitable, and the reflection of the film is assumed to be zero. Since  $T_f^2$  is small, the term  $\log(1 - R_1 R_s T_f^2)$  may be neglected, so that with the gray film in place  $E_{pf1} = E_p$ , the desired true absorbance of the sample.

In an exactly similar way, the true absorbance  $E_d$  for diffuse incident light may be measured by placing the opal glass in front of the sample instead of behind it. The change of measured absorbance due to the effect of the multiple reflection is found to be small as compared with the height of the bands of the spectrum. When the incident light is parallel and the sample (e.g. an algal suspension) reflects relatively little, this effect is negligible. Even when the sample is highly reflecting, no distortion or shifting of bands due to this effect is observed. The only effect is on the heights of the bands and of the base line.

By illuminating the sample through an opal glass on the detector side, it is possible to measure the diffuse reflectance in ab-

solute terms without comparison with a standard. A device for these measurements has been made to fit into a recording spectrophotometer. Its operation is shown in figure 6. The beam (shown by the dotted arrow) which is specularly reflected from the front surface of the opal glass does

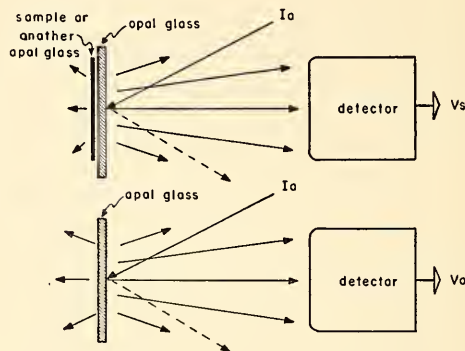


FIG. 6. Measurement of diffuse reflectance

not reach the detector. The other terms used are defined as follows:

- $I_0$ , intensity of incident parallel light;
- $I_1$ , intensity of diffusely reflected light from a single sheet of opal glass;
- $I_{1+s}$ , intensity of diffusely reflected light from the opal glass with the sample behind it;
- $I_2$ , intensity of diffusely reflected light from the opal glass with another opal glass behind it;
- $T_1$ , transmittance of the opal glass for diffuse incident light;
- $T'_1$ , transmittance of the opal glass for parallel light falling on the surface at the angle of incidence used;
- $R_1$ , reflectance of the opal glass for diffuse incident light;
- $R_s$ , reflectance of the sample for diffuse incident light;
- $R'_1$ , reflectance of the opal glass for parallel light falling on the surface at the angle of incidence used.

Now:

$$I_{1+s} = I_0 \left( R'_1 + \frac{T'_1 T_1 R_s}{1 - R_1 R_s} \right),$$

$$I_2 = I_0 \left( R'_1 + \frac{T'_1 T_1 R_1}{1 - R_1^2} \right),$$

$$I_1 = I_0 R_1',$$

$$D_{1+s} = I_{1+s} - I_1 = \frac{I_0 T_1' T_1 R_s}{1 - R_1 R_s},$$

$$D_2 = I_2 - I_1 = \frac{I_0 T_1' T_1 R_1}{1 - R_1^2}.$$

Therefore

$$\frac{1}{R_s} = R_1 + \frac{D_2}{D_{1+s}} \left( \frac{1}{R_1} - R_1 \right).$$

where

$V_{1+s} = V_s/V_0$  for the opal glass backed by the sample in the sample beam;

$V_2 = \bar{V}_s/V_0$  for two sheets of opal glass in the sample beam;

$V_1 = \bar{V}_s/V_0$  for one sheet of opal glass in the sample beam.

The quantity plotted by the recorder is  $V_s/V_0$ . For the above three types of re-

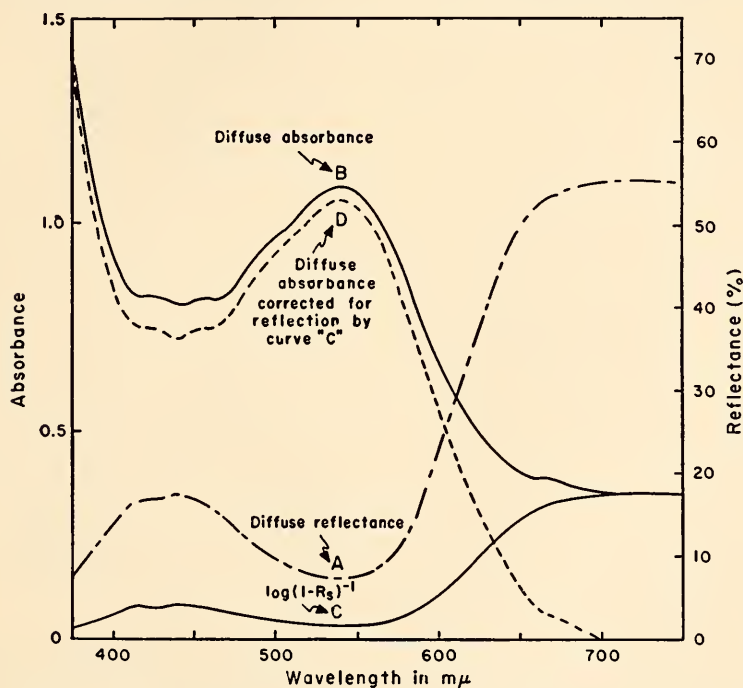


FIG. 7. Relation between the diffuse reflectance and true absorbance spectra of Coleus leaf. Curves *A*, *B*, *C*, and *D* are spectra in diffuse reflectance ( $R_s$ ), diffuse absorbance ( $E_d$ , corrected for multiple reflections),  $\log(1-R_s)^{-1}$ , and  $E^*$  units, respectively. The scale on the right is for curve *A*.

Here,  $R_s$  is obtained from the ratio  $D_2/D_{1+s}$  and the diffuse reflectance of opal glass,  $R_1$ . In practice,  $R_1$  is determined for the actual sample of opal glass by analogous measurements with one and two sheets of opal glass and a gray film. A table of these values for the visible spectrum and the detailed method of their measurement is being published elsewhere.  $D_2/D_{1+s}$  is obtained from the detector output voltages as follows:

$$\frac{D_2}{D_{1+s}} = \frac{I_{1+s} - I_1}{I_2 - I_1} = \frac{V_{1+s} - V_1}{V_2 - V_1},$$

ording, there is an opal glass in the reference beam. In this way, curves are recorded from which the diffuse reflectance is calculated.

As examples, spectra of a variegated Coleus leaf are shown in figure 7. Curves *A* and *B* are total diffuse reflectance ( $R_s$ ) and absorbance ( $E_d$ ) spectra of the leaf, respectively. In order to obtain the relation between these spectra, a new quantity ( $E^*$ ) was defined as  $\log(I_0 - I_r)/I_t$ , where  $I_0$ ,  $I_r$ , and  $I_t$  are the intensities of incident, reflected, and transmitted light, respec-



tively.  $E^*$  is an idealized quantity in absorbance dimensions, which would be obtained if the sample transmitted the light in the same way as the actual sample did, but did not reflect the incident light at all. As is clear from the following equation,  $\log(1-R_s)^{-1}$  has to be subtracted from  $E_d$  to obtain  $E^*$ :

$$\begin{aligned} E^* &= \log(I_0 - I_r) / I_t \\ &= E - \log(1 - R_s)^{-1}. \end{aligned}$$

The spectrum in  $\log(1-R_s)^{-1}$  units is shown by curve *C*. Where there is no absorption band beyond 700 m $\mu$ , curves *B* and *C* coincide. Therefore, the base line of the spectrum in  $E^*$  units (curve *D*) is the actual zero line. This means that the incident light is either transmitted or reflected in the region of no absorption. This is a proof that the methods of using opal glass plates for measurements both of diffuse absorbance and of diffuse reflectance give the correct values. Therefore, we can obtain the correct value of absorption at the wavelengths absorbed by the pigments in the sample.

When the value of reflectance is low, the shape of the curve gives little information about the pigments concerned. For studying absorption spectra, the absorbance,  $\log(1/T_s)$ , is used, since it gives curves of the same shape differing from one another only by a constant factor determined by the pigment concentration. A corresponding quantity,  $\log(1/R_r)$ , where  $R_r$  is the relative reflectance of a sample, is proposed for use with reflection spectra. This very useful quantity may be recorded more simply than the reflectance itself. Measurements of this sort are made as described (see figure 6), but an opal glass plate is used in the reference beam only, and the logarithm of the voltage ratio is recorded.

Among various samples observed by this method, the spectra of carotenoids in their natural states showed interesting bands. First, the spectra of crystalline  $\beta$ -carotene and lycopene were observed. The results indicated that the spectra have an additional band for each carotenoid besides the

bands which correspond to those in spectra of their solutions. This additional band was identified in the relative reflectance spectra of intact plant materials. The additional band of  $\beta$ -carotene in carrot root was found at 515 m $\mu$ , and that of lycopene in the fruits of watermelon, tomato, and a pink variety of grapefruit was found at 562 m $\mu$ . Some carotenoids, therefore, exist in crystalline form in their natural states, a fact which has previously been suspected from microscopic observations.

Including the previously developed method of observing absorbance, we have now three methods of using opal glass that give us the correct values of absorbance, diffuse reflectance, and  $\log(1/R_r)$ . From absorbance and diffuse reflectance, the amount of the light absorbed by a sample (true absorption) can be calculated. The advantage of these methods is not only the absoluteness of the values obtained but also the simplicity of the methods, which enable us to use commercially available spectrophotometers with only a small attachment for installing opal glass plates. Above all, by these methods samples can be observed in their intact states. Combinations of the methods may be used as a standard technique in the spectroscopy of translucent materials.

#### DERIVATIVE SPECTROPHOTOMETRY

C. STACY FRENCH, BRUNO F. LUDOVICI, AND  
GORDON E. HARPER

Minor constituents of pigment mixtures are often difficult to detect by inspection of curves of absorbance plotted against wavelength, when the minor band occurs on a steep side of a large band, thus showing only as a slight inflection. The first derivative of an absorbance curve gives a continuous measure of its slope. In first derivative curves the side of a major band comes out as a more or less horizontal line. Even a small band shows clearly on a first derivative plot as a positive and a negative peak, so the detectability of the minor band is practically independent of the presence



of an overlapping large band. The second derivative shows the slope of the first derivative curve or, in other words, the curvature of the absorbance curve. The expected advantages of derivative spectroscopy over direct absorbance measurements for the detection of trace amounts of pigments have been discussed in the last two annual reports. Derivative measurements appear to be of particular value in the study of pigments in living cells.

During the report year the work in progress by Mr. F. Singleton of the Graesser Chemical Works (Sandycroft near Chester, England) on derivative spectrophotometry was brought to our attention, and a visit was made to his laboratory. Mr. Singleton has done work of extraordinary precision with direct recording of the second derivatives of absorption spectra for the analysis of mixtures of cresols. The apparatus used by Singleton first produces an electrical signal proportional to light absorption. The signal is then twice electronically differentiated with respect to time. Such an arrangement requires extreme stability of the entire apparatus and the use of a highly constant speed for the wavelength sweep. These requirements appear to have been met, and the instrument has given excellent results.

We have nevertheless preferred to continue with the development of our apparatus, which directly produces an electrical signal proportional to the first derivative by use of a vibrating slit. The reason for this choice is that the direct generation of an a.c. signal, itself proportional to the derivative, eliminates the need for electronic differentiation and for the extreme stability of the apparatus which this entails. In theory, the vibrating slit appears to be capable of equal accuracy with greater simplicity, or conversely of greater accuracy with the same degree of complexity of the apparatus, than does a system dependent on electronic differentiation.

Very recently a method has been proposed by Dr. J. P. Pemsler of Nuclear Metals, Inc., Cambridge, Massachusetts, for

recording first derivative spectra with a commercially available double-beam spectrophotometer which has its two beams at slightly different wavelengths. There is no information yet as to the performance or sensitivity actually attained with this method. The field of derivative spectrophotometry, being in its infancy, has not yet developed to the point where quantitative measures of performance have been compared.

During the early part of this report year our equipment was greatly modified by Dr. Strehler for another purpose: the measurement of difference spectra of cellular suspensions. The results are described in a separate section of this report. When the work of Drs. Strehler and Lynch was completed, the apparatus was redesigned, and it is now being rebuilt to record derivative spectra in an improved way. The apparatus we described last year required the measurement of four separate curves from which the first derivative spectrum could be calculated. The present system should plot the derivative of absorbance directly.

The new system uses the previously described slit, vibrating at 100 cycles per second between two adjacent wavelengths a few millimicrons apart in the spectrum. After passing through the vibrating slit, the beam hits a half-circular mirror rotating at a constant speed of approximately 10 rps. The mirror alternately sends the beam through a reference cell and through the sample cell. During the period when the beam is going through the reference cell, an adjustment is made automatically so that during the other period when the beam is directed to the sample cell the measurement of the sample characteristics can be performed. On the shaft supporting the rotating mirror there are three cams which operate switches to send the photomultiplier voltage to the appropriate electrical channels. Thus during the reference period the signal is sent to the servo amplifier to control a diaphragm in the optical system. During the measurement period the signal goes to the recording system.

From the rotating mirror the light passes through a cell containing the reference material with which the sample is to be compared. After going through the reference cell the beam is diffused by opal glass and enters a light-mixing chamber lined with magnesium oxide. This chamber is arranged so that the photomultiplier is illuminated in the same way by a beam through the reference cell as it is by a beam through the sample cell. The new chamber should have a higher light-gathering efficiency than the white sphere previously used.

As the slit vibrates between two wavelengths a few millimicrons apart in the spectrum, the light on the photomultiplier varies in intensity during each cycle in proportion to the intensity of the two wavelengths. Thus the photomultiplier puts out an alternating voltage proportional to this difference in intensity and of the same frequency as that of the slit vibration. This 100-cycle voltage is a measure of the derivative with respect to wavelength of the effective spectral intensity curve for the apparatus without the sample. The shape of the effective spectral intensity curve depends on the spectral emission of the tungsten lamp, the monochromator transmission, the spectral sensitivity of the photomultiplier, the variation with wavelength of the reflection of the mirrors, and the transmission of the lenses. The derivative as well as the height of the effective spectral intensity curve of the apparatus without the sample must be continuously controlled during the measurement. The 100-cycle voltage generated by the reference beam is amplified and drives a servo system that positions a diaphragm partly obscuring the spectrum on one side of the center of travel of the vibrating slit. The servo reduces the 100-cycle voltage, and hence the derivative of the effective spectral intensity curve, to zero.

When the rotating mirror is out of the beam, the light hits a stationary mirror just behind the rotating mirror. The reflected beam then passes through a 600-

cycle light chopper and goes through the sample cell to the light-mixing chamber and the photocell. Since the diaphragm on one side of the vibrating slit has been appropriately positioned by the servo under the control of the reference beam, the difference in intensity of the two adjacent wavelengths of the sample beam before it passes into the sample has been adjusted to zero. Therefore any difference of intensity between the two adjacent wavelengths after the beam goes through the sample must be caused by differential absorption of these two wavelengths within the sample. In this way the necessity for making a control record for the base line to which the derivative of the sample transmission is referred should be eliminated. In our previous work we found that the base line went off the scale of the recorder within a narrow wavelength range when an adequately high sensitivity was used. Therefore this automatic zero adjustment should be of considerable practical value.

In order to obtain the true value of the derivative of the sample absorbance with respect to wavelength, it is not sufficient merely to have the base line for the measurement fixed. Two other factors must also be considered. One of these is the variation of the effective light intensity with wavelength, that is, the height of the effective spectral intensity curve. The other is the transmission of the sample itself for light of the average wavelength in the small wavelength interval over which the derivative is measured. Fortunately, these two factors, the height of the spectral intensity curve and the sample transmission, appear in the expression for the derivative of the absorbance as the product of the two quantities. A single value proportional to the product of these two quantities can correct simultaneously for both variables. The total effective light intensity of the two adjacent wavelengths passing through the sample and detected by the photocell is proportional to this desired quantity. In order to measure this correction factor and the derivative signal simultaneously, fre-

quency selectivity is employed to separate the two signal voltages. The vibrating slit has a frequency of 100 cps, while the entire beam passing through the sample cell is modulated by a 600-cps light chopper. The electrical signals of these two frequencies are separated by tuned amplifiers during the half cycle of the rotating mirror that passes the light beam through the sample. One tuned amplifier produces an output proportional to the 100-cycle signal, which is a measure of the difference in absorption for the two adjacent wavelengths. The 600-cycle-tuned amplifier simultaneously gives an output proportional to the effective spectral intensity multiplied by the sample transmission. After rectification and filtering, the ratio of these voltages is plotted by a voltage-ratio recorder to give a deflection proportional to the first derivative of absorbance. Thus the need for recording a separate curve for each of the necessary quantities is eliminated.

Most of the new construction has been completed and the various component assemblies are being tested and modified. It is still too soon to be sure that the present system will be satisfactory. No serious difficulty has yet appeared, although numerous minor troubles have so far prevented an operating test of the complete system. The present apparatus is more stable than the model previously described and appears to operate adequately for point-by-point measurements. The major difficulties remaining lie in the achieving of adequate sensitivity of response of the servo system so that records can be made by sweeping through the spectrum automatically.

#### PHOTOTAXIS IN FLAGELLATES

PER HALLDAL

The flagellates constitute a significant fraction of the marine plankton which convert light into fish food. These organisms will swim either toward or away from the light, the direction depending on their recent past environment in ways that are far from being understood. Halldal has

previously investigated the effect of light and the effectiveness of different wavelengths of light arousing this phototaxis at the University of Oslo, Norway, at the Physical Institute of the State University, Utrecht, Holland, and at the Hopkins Marine Station, Pacific Grove, California. During the past year, spent at the Department of Plant Biology, the work has been continued on the following lines: More definitive action spectra of phototaxis have been obtained; the pigments of the organisms have been studied, particularly in respect to their spectral absorption, in an effort to identify the photoreceptor for the phototactic reaction; and attempts have been made to identify the substance or substances which control the direction of movement of the organisms in light.

Two methods for measuring the action spectra of phototaxis have been used. One method depends on counterbalancing the movements of the organisms by placing them in two opposing light beams of equal effectiveness. One beam, the reference beam, is kept constant in wavelength and intensity; the other, a monochromatic beam, is varied in intensity until the organisms swim to neither light source. By measuring the intensity of a series of monochromatic beams, the relative phototactic effect of different wavelengths can be determined, and thus the data for the action spectrum of phototaxis are obtained. The other method measures the action spectrum with a single exposure, by a scheme diagrammed in figure 8. A vessel containing a suspension of the organisms is illuminated from one side with a reference light uniform in intensity, and from the other side with a projected spectrum having an intensity gradient at a right angle to the spectrum. Under these light conditions the organisms will collect on the side of the vessel illuminated by the spectrum wherever this light is more effective than the uniform reference beam. If the horizontal spectrum is adjusted to produce equal numbers of quanta at each wavelength and the vertical gradient is known,



the upper limit of collection will represent the action spectrum of phototaxis for this organism.

The action spectra of phototaxis have been determined by these two methods for the following species:

Vovocales:	Isolated by
Dunaliella salina .....	A. Gibor
Dunaliella viridis .....	A. Gibor
Platymonas subcordiformis .....	A. Gibor
Stephanoptera gracilis .....	A. Gibor
Dinoflagellatae	
Goniaulax catenella .....	P. Halldal
Peridinium trochoideum .....	E. Nordli
Prorocentrum micans .....	E. Nordli

In our attempts to correlate action spectra with absorption spectra of pigments, we have not been able to show the presence of any pigment with an absorption spectrum corresponding to an action spectrum of phototaxis. *Peridinium* and *Prorocentrum*, which have quite different action spectra of phototaxis, have been subjected to analysis. This work was performed mainly at the University of Oslo. The absorption spectra of living *Peridinium* and *Prorocentrum* were recorded and the fat-soluble pigments were extracted and separated by means of two-dimensional paper chromatography. The absorption spectra of live cells of *Peridinium* and *Prorocen-*

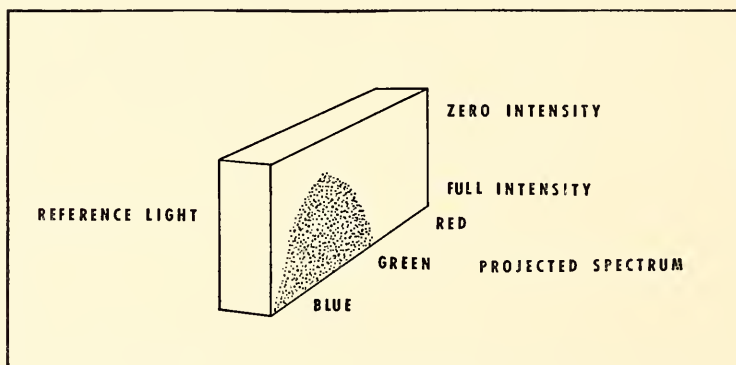


FIG. 8. Method of measuring the action spectrum of phototaxis in a single exposure

The action spectra of phototaxis in *Platymonas subcordiformis*, *Peridinium trochoideum*, and *Prorocentrum micans* are shown in figure 9. The action spectra for the *Dunaliella* species and for *Stephanoptera* are similar to that for *Platymonas*, and that for *Goniaulax* is the same as that for *Peridinium*.

In *Platymonas* the phototactic response lies between 400 and 570 m $\mu$  with a maximum at 493 m $\mu$  and a small shoulder at 435 m $\mu$ . *Peridinium* and *Prorocentrum* differ greatly in their phototactic response. *Peridinium* shows reaction from 400 to 540 m $\mu$  with a maximum at 475 m $\mu$ , whereas *Prorocentrum* reacts to light of wavelengths from 480 to 640 m $\mu$  with a maximum at 570 m $\mu$ .

*trum* did not show any qualitative difference, and the same pigments were obtained for both species on the paper chromatograms. This indicates that the pigments involved in the phototaxis of these two species are present in very small amounts in the cells and that none of the major carotenoids participates directly.

When any of the organisms examined is transferred to fresh medium, a negative phototactic reaction occurs immediately. The direction of response is independent of light intensity. If the organisms are kept for some hours in the same medium, their reaction becomes positive. Their movement toward the light is independent of intensity. Some experiments have been performed with *Platymonas* to analyze the



chemical factors affecting this reaction. It has been shown that  $\text{CO}_2$  and  $\text{O}_2$  are not primarily involved in the change. The change to a negative reaction occurs when *Platymonas* is transferred to artificial sea water consisting of a solution of nine of the major salts present in the sea. It is not caused by 2.5 per cent NaCl in distilled water. However, 0.1 per cent  $\text{CaCl}_2$  in the 2.5 NaCl solution does change the reaction

a negative reaction in *Platymonas* has also been produced with indole-3-acetic acid.

#### THE GROWTH OF ALGAE IN CROSSED GRADIENTS OF LIGHT INTENSITY AND TEMPERATURE

PER HALLDAL AND C. STACY FRENCH

The general plan of studying the response of living organisms exposed on a plane surface to a continuous gradient of

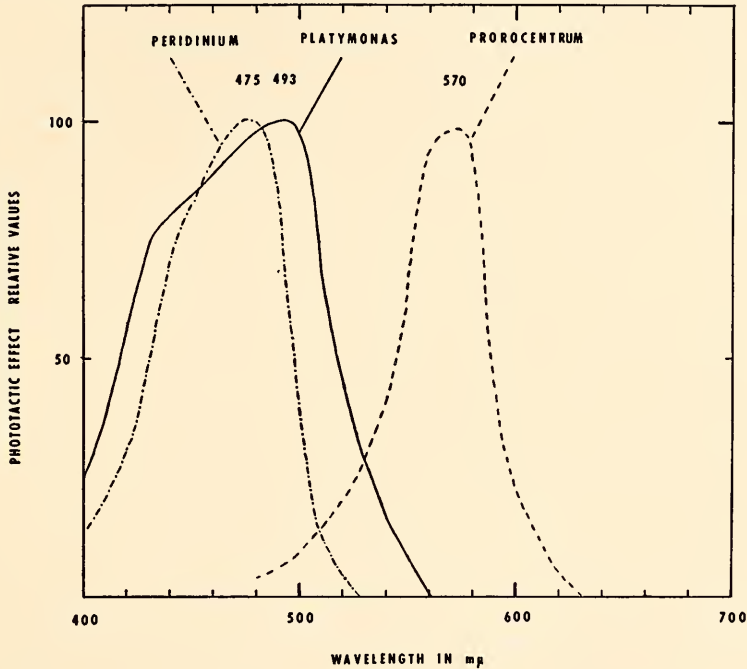


FIG. 9. Action spectra of phototaxis in *Platymonas subcordiformis*, *Peridinium trochoideum*, and *Prorocentrum micans*

from positive to negative, and in this solution the reaction stays negative for several days. None of the other major elements in sea water produces this change. The optimum concentration of  $\text{CaCl}_2$  obtained is about 0.05 M (in 2.5 per cent NaCl). The effect is found only within the range of concentration of 0.005 to 0.3 M  $\text{CaCl}_2$ . The same effect has been produced with other calcium salts. Therefore, calcium evidently is involved in the phototaxis of *Platymonas*. A change from a positive to

some relevant variable is widely followed in biological investigations. This method is particularly useful with microorganisms because of their small size and their capacity for growth on the surface of agar. A well known application of this principle is the agar plate assay for antibiotics. The test for utilization of nutrients by bacteria made by dropping individual crystals of various substances on an inoculated plate of agar containing the other required nutrients also applied this idea. The spec-

trum projection experiments of Engleman around 1888 were one of the earlier and, for that time, one of the more elegant uses of the principle in which the gradient was the wavelength of light and the plant response was evidenced by photosynthetic activity or by the aggregation of photosynthetic bacteria at the absorption bands of the active pigments.

Recently H. I. Virgin and also Halldal have used a more refined modification of Engleman's procedure for quantitative measurements of action spectra in living plants. One refinement was the measurement or compensation of the intensity gradient accompanying the wavelength gradient. The major improvement was the simultaneous use of a gradient of another variable at right angles to the first variable. In their work, wavelength was varied along one axis and the light intensity of each wavelength along the other axis. The position corresponding to threshold response of the organisms exposed to the crossed gradient fields gave a direct determination of the action spectrum for the response observed. The work in progress on such action spectra is discussed in the preceding section of this year's report.

We have attempted to use the two-dimensional field principle in the study of algal growth. This method gives a quick way of finding the optimum light intensity and temperature for laboratory cultivation of any organism that will grow on an agar surface or submerged within an agar film. In the course of the work it became evident that the method can be used for a number of different kinds of experiments in algal physiology.

The apparatus illustrated in figure 10 consists of a  $\frac{3}{4}$ -inch-thick aluminum alloy plate 12 by 16 inches in size. One end of the plate is heated and the other end cooled by pumping water from thermostats through holes in the ends of the plate. An 11-by-12-inch part of the plate, the growth chamber, is painted with chemically inert white Tygon and has raised metal edges  $\frac{1}{8}$  inch high which support a Lucite cover.

Across the growth area there is a smooth temperature gradient in one direction. At right angles to the temperature axis there is a light-intensity gradient varying almost linearly from about 1000 to about 25 foot-candles. Along any line parallel to the temperature axis the light intensity is approximately constant. Two 300-watt reflector spot lamps and one 150-watt reflector flood lamp are placed 27 inches above the growth area near the high-intensity end of the light-gradient axis. The infrared is removed by a  $4\frac{1}{2}$ -inch-deep layer of water cooled by a fan. The light-intensity gradient is produced by a ground-glass screen partly covered with a light-weakening comb made of translucent white paper, and is adjusted by appropriate shaping of the comb and by positioning the lights. To achieve sufficiently low intensities at the end of the intensity axis, a black paper shade is placed 2 inches above the growth surface, extending 3 inches over the lower end of the intensity axis. The illumination thus produced on the growth surface is diffuse. Its intensity is measured by a photocell moved around in the plane normally occupied by the agar surface. The temperature is measured with a thermocouple taped to the under surface of the metal plate or stuck into the agar. A continuous slow flow of 5 per cent  $\text{CO}_2$  in air after bubbling through water enters six ports along the front edge of the plate. Each port is made of an aluminum tube containing a roll of wet blotting paper, held at approximately the same temperature as the adjacent part of the metal plate by heat conduction through the aluminum walls. Thus each air stream is approximately saturated with water vapor at the appropriate temperature for the place at which it enters the growth chamber. The air passes along a line at the same temperature and emerges through slots under the Lucite cover at the opposite side of the chamber. At first the agar dried out rapidly on the high-temperature side of the chamber, the water condensing on the Lucite cover. This effect was diminished but not entirely eliminated

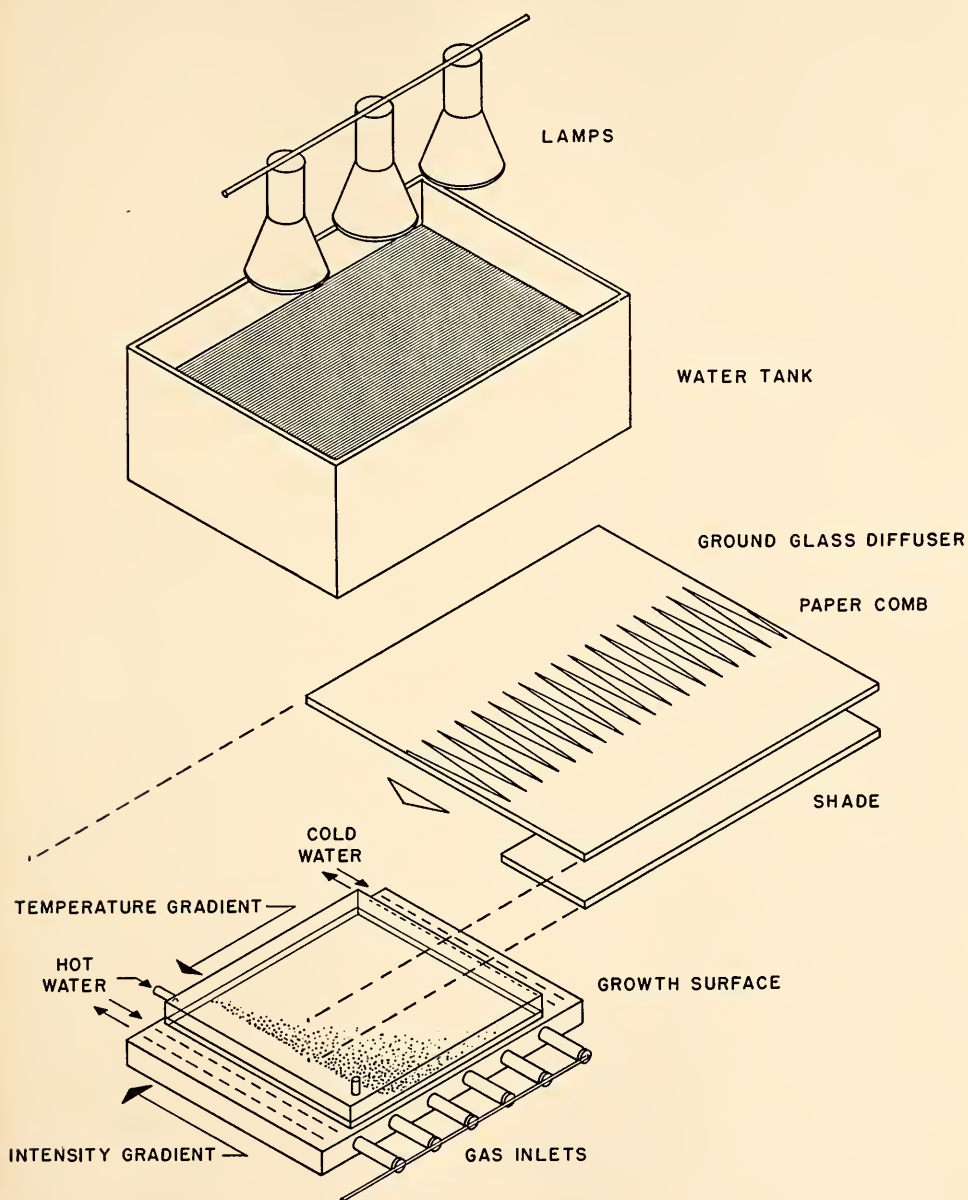


FIG. 10. Apparatus for growing algae in crossed gradients of light intensity and temperature

by making the cover a shallow Lucite box through which warm water is circulated.

At the start of an experiment the metal plate is removed, leveled, and warmed to 50° C. Hot agar containing a salt nutrient mixture is poured on and allowed to cool. A suspension of the algae is flooded over the whole surface and allowed to stand over night. The excess liquid evaporates or is taken up by the agar. Spreading the algae by painting or spraying was found less satisfactory. In some cases it is necessary to start the algal growth with the whole plate at room temperature and with uniform weak illumination. When a uniform thin layer of growing algae has been established, the temperature and intensity gradients are set up. In a few days the culture has a strongly different appearance in different parts of the chamber. At a sharply defined upper temperature the growth stops at all light intensities, and above this temperature the color is completely bleached. The region of moderate growth is often L-shaped, being more or less independent of intensity over an intermediate part of the temperature range and more or less independent of temperature over a moderately low part of the intensity range. The best growth takes place where these broad lines intersect at an intermediate temperature and moderately low intensity. Photographic records are made at intervals with a camera fixed to the supporting frame of the apparatus.

For *Chlorella pyrenoidosa*, Emerson's strain, the maximum temperature for growth was 31° C, the region of optimum growth between 22° and 27° C and from 100 to 300 f.c. This limit of growth at higher temperature is appreciably lower than has been found in liquid cultures of the same strain. With the high-temperature *Chlorella* species of Myers and Sorokin, Tx 71105, the temperature limit was 43° C and the optimum range 28° to 39° C and 100 to 300 f.c. With a blue-green alga, *Anacystis nidulans*, of Kratz and Allen, which had an upper limit for growth at 46° C and an optimum at 30° to 40° C and 100

to 400 f.c., there was a striking difference of color in different parts of the growth area. The algae were blue, presumably owing to greater phycocyanin formation, in a region roughly from 35° to 46° C and from 100 to 600 f.c. In the high-intensity region at low temperature they were a muddy yellow-green, and at low temperature and low intensity a somewhat deeper green. Attempts are being made to record the appearance of such cultures on color film and to make spectrophotometric determinations of the pigment production in various areas. The study of environmental factors in pigment formation by the crossed-gradient method may turn out to be even more promising than the study of algal growth itself.

For the present we have made only qualitative observations of growth or of pigment formation in the various areas on the crossed-gradient plate. It seems feasible, though hardly necessary for exploratory purposes, to make quantitative measurements of growth or of pigment formation by removing the algae from equal sample areas in various parts of the plate for measurement by cell counts or pigment analysis. The crossed-gradient plate should facilitate the investigation of many aspects of algal physiology and microorganism ecology besides those dealt with in the present experiments on the patterns of growth and of pigment formation of common algae. It should be possible to isolate new strains of algae suitable for growth under various combinations of temperature and intensity by inoculating the plate with a natural source of a mixed algal population. Such an experiment should also yield information as to the relative abundance, in natural samples, of algae adapted to specific combinations of conditions.

Inoculation of the plate simultaneously with nearly equal numbers of algae of two species would show the conditions under which one would outgrow the other. Information of this type may perhaps have some practical importance in adjusting the growth conditions in a large-scale algal cul-



ture to avoid the overgrowth of a desired alga by a contaminant. Since effects of this type may be different on an agar surface and in liquid culture, it may be desirable to adapt the crossed-gradient principle to liquid culture. This could be done, though with some loss of resolution, by making many shallow holes in the plate each of which would contain an independent culture exposed to a slightly different environment as provided by the over-all intensity and temperature gradients on the plate. The use of different nutrient solutions in the different holes should provide an easy way to combine a graduated series of nutrient solutions with an in-

tensity or temperature gradient. Though the plate we have used was made primarily for microscopic algae, the same principle appears to be applicable to studies on small higher plants such as the duckweeds whose growth rates as a function of temperature were reported in the last two Year Books by Dr. Elias Landolt.

The results to date suggest the following combinations of temperature and intensity of tungsten light for the most reliable growth on agar of the algae studied so far: *Chlorella pyrenoidosa*, Emerson strain, 25° C, 200 f.c.; *Chlorella* sp. Tx 71105, Sorokin and Myers, 35° C, 200 f.c.; *Anacystis nidulans*, Kratz and Allen, 40° C, 300 f.c.

## PALEOBOTANY

RALPH W. CHANEY

Since the appearance of land plants several hundred million years ago, the forest cover has been intimately related to the history of life on the continents, and has widely affected the processes which shape the earth's surface. In later ages the appearance of modern types of plants has provided a basis for comparisons with forests of today, and for the reconstruction of ancient terranes whose climate and topography are judged to have been similar to those of corresponding units of living vegetation.

In any such projection of existing forests into the past, it is desirable to know not only what kinds of trees lived in them, but also whether the various tree species were abundant or rare. Many fossil floras are too small to provide adequate answers to these questions, but the Tertiary vegetation of the Columbia Plateau is so richly represented in the volcanic sediments of the John Day Basin that it is possible readily to learn the essential facts regarding many of the forests which have lived there during past tens of millions of years.

The 1956 field season afforded an opportunity to collect and identify a large number of fossil leaves and fruits from the

Lower John Day formation near Twickenham, Oregon. This Oligocene flora has for many years been studied at near-by localities, where it is characterized by foliage and cones of the Tertiary Chinese redwood, *Metasequoia occidentalis*, together with the leaves and fruits of deciduous angiosperms. But at no locality previously studied has *M. occidentalis* been so dominant a member of the Oligocene forest. Here its foliage shoots and cones make up nearly 85 per cent of all the fossil specimens counted, which reached a total of 35,000. There is little question that this tree once formed a pure stand at Twickenham similar to those of the living coast redwood (*Sequoia sempervirens*) on stream flats near the coast of northern California.

By contrast, the living Chinese redwood, *M. glyptostrobooides*, does not now hold a similarly dominant place in the mountain valleys of central China. Though in some sites more abundant than any other tree, it nowhere appears to make up more than one-fourth of the population. If there were wider areas where its modern occurrence could be studied, we might find the Chinese redwood as abundant in some places as was its ancestor in the Tertiary forest

at Twickenham. This Oligocene record serves to amplify our knowledge of a tree today on the verge of extinction.

Other trees growing with the Chinese redwood have Tertiary equivalents in the rocks of Oregon. One of the rarer of these, though of regular occurrence, is the katsura (*Cercidiphyllum japonicum sinicum*), of which no more than one tree has been found growing in any of the valleys visited. The low score, 0.2 per cent, of its fossil equivalent, *C. crenatum*, in the Twickenham forest indicates that it also was a rare tree. The specimen count of the fossil sour gum, a species of *Nyssa* as yet undescribed, shows that it was no more numerous than are trees of this genus now living with *Metasequoia*. Oaks, birches, and maples are also rare in both the fossil and the modern forests. Chestnut, one of the most common associates of the redwood in central China, has yet to be recorded in the Twickenham flora, though leaves almost identical with those of a living Asiatic species are numerous at another fossil locality 35 miles to the southeast. Alders are second in number to *Metasequoia* in the fossil record, but have not been seen in immediate association with the redwood in China. Such differences are to be expected between two forests separated by so wide a span of time, and make all the more remarkable the similarities in composition which have been noted in previous annual reports. A detailed analysis of these quantitative data is now being made, and will be discussed at length in future reports on this Oligocene assemblage, commonly known as the Bridge Creek flora.

Recent studies based on smaller, but statistically adequate, specimen counts from Miocene rocks in the John Day Basin have provided illuminating evidence of the altitudinal occurrence of several fossil conifers. Firs and spruces are today found more commonly in mountain forests of the western United States than in the lowlands.

If they had a similar occurrence in the Miocene, we might expect their remains to be less numerous in the fossil record than those of trees which lived at lower elevations. Their organs must have been transported for greater distances before they were deposited along with volcanic sediments in the lowlands, and the hazards of such a journey must have greatly reduced their representation. Such trees as beech, oak, birch, and maple commonly live on the borders of lakes and rivers, where their leaves and fruits may readily enter sedimentary deposits; fossil specimens of these trees are among the most abundant in the record of the Miocene flora of the Blue Mountains, making up three-fourths of all the specimens collected. By contrast firs and spruces comprise only 1.25 per cent, and their winged seeds are much more commonly found than their foliage branchlets and cones. If these conifers lived on distant slopes during the Miocene as they do today, we should expect to uncover relatively few fossil specimens, and to find their readily wind-borne seeds more common in the record than heavier units. This is but one of many applications of quantitative analysis in the study and interpretation of plant populations of the past.

It is obvious that to make such inferences accurately, we must have a thorough knowledge of the present occurrence of trees and shrubs, and also of the manner and rate at which their leaves and fruits are now accumulating. Studies of contemporary plant deposition, long under way, have during the past year been extended to Japan, where volcanic sediments like those of the Tertiary are being widely deposited, and where modern forests are more similar to those of the Miocene and Oligocene than in any other part of the world. A fuller knowledge of these forests and of their record in the sands of today should provide a fuller understanding of fragmentary remains of yesterday.

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## DEPARTMENT OF EMBRYOLOGY

*Baltimore, Maryland*

JAMES D. EBERT, *Director*

Even the casual observer of research in the biological sciences today is struck by the resurgent interest in the mechanisms of development. In the field of embryology at this time forces are being regrouped and new weapons are being forged to attack the fundamental problems of differentiation, growth, and morphogenesis. These problems, always challenging, are today more exciting than ever before as they become amenable to analysis with a variety of new tools. Methods of disaggregation of tissues offer opportunities for isolation and recombination experiments at the cellular level, leading to new approaches to a number of questions, including the origin and role of cell and tissue affinities, and the significance of critical mass in the stabilization of cell types. The use of clonal lines of cells presents a challenge to those who would explore the applicability of the concept of genetic transduction to the field of embryonic differentiation. The availability of cellulose ester membrane filters of varying porosities for explantation and transplantation techniques promises new information concerning the mechanisms of embryonic induction and the nature of the immune reaction to homografts. The techniques of biochemistry and physical chemistry combined with modern optical methods permit the focusing of attention on the fine structure of cells and their products, and on the mechanisms by which chemical energy is generated and transferred to the molecular lattice of the cell. Studies of subcellular structures are still in their infancy: there is considerable controversy about the methods to be used and the results obtained. These problems require the skills of both the biochemist and the morphologist, at present particularly those of the latter. It is against this background that we consider the activities

of the Department of Embryology for the past year.

The year 1955-1956 has been a period of eventful change in the composition of the scientific staff of the Department. Dr. George W. Corner, widely known for his scientific acumen, critical judgment, and clarity of presentation in these annual reports, as well as in numerous other writings, retired from the directorship on December 31, 1955, after sixteen years of service. In his final annual report (Year Book No. 54), Dr. Corner looked back over forty-two years to summarize the accomplishments of the Department in fulfilling the objectives set forth by Franklin P. Mall in his "Plea for an Institute of Human Embryology." In his account, he modestly refrained from emphasizing one point, which is clear to the discerning reader, namely that a significant part of the progress was achieved under his own leadership. Under the first two directors, Franklin P. Mall and George L. Streeter, the emphasis in the Department had been placed on morphology, although morphology was not the sole preoccupation. Under Dr. Corner's guidance, a gradual shift of emphasis aided in directing attention to biochemical and biophysical approaches to problems of embryology and mammalian reproduction, while holding fast to the solid achievement in morphology.

Upon his retirement, Dr. Corner left the imprint of his talent not only in the record of his personal investigations, which have been summarized in these Year Books, but also in the broad outlook and vigor of the Department. His achievements in the biological and medical sciences and as historian and humanist have been described in detail by Dr. Carl G. Hartman and Dr. Richard H. Shryock in volume 98 of the

*American Journal of Anatomy*, published as *The Corner Festschrift Volume*. In these articles, Dr. Corner's accomplishments have been delineated with understanding and wisdom. I would add only one comment: not the least tribute that can be paid Dr. Corner lies in the fact that he welded together in the Department of Embryology a scientific and technical staff whose loyalty to the Department equaled its loyalty to him as a person. Hence, significant research has been carried forward by the established members of the staff in spite of the difficulties always presented by reorganization of activities and renovation of physical plant. The members of the Department are pleased that Dr. Corner will be able to continue his work as a member of the Rockefeller Institute for Medical Research, where he has facilities for research in both experimental biology and medical history. On January 1, 1956, he was appointed Professor of Embryology Emeritus in the Johns Hopkins University.

Dr. Samuel R. M. Reynolds, Staff Member, was invited to head the Department of Anatomy of the Medical School of the University of Illinois and resigned on December 31, 1955. Although Dr. Reynolds, who was appointed to the staff first in 1941, has as his major objective the study of the physiology of the uterus, his investigations demonstrate his remarkable breadth of interests and depth of understanding in the fields of mammalian reproduction and fetal physiology. To his research he brings unbounded determination and energy and great technical skill. His achievements, which have been documented in these Year Books, have brought him to a position of eminence in his field. It is gratifying that Dr. Reynolds will continue his connection with the Department of Embryology as Research Associate.

Dr. Arpad I. Csapo, who has been associated with the activities of the Department since 1949 as Fellow, Research Associate, and later Staff Member, was appointed an Associate Member of the Rocke-

feller Institute for Medical Research on January 1, 1956. While a member of the staff, Dr. Csapo centered his attention on problems of uterine chemistry and physiology with special emphasis on the contractile proteins of the uterus. His experiments in that field, initiated in collaboration with Dr. Corner, have opened a broad avenue for future research.

Mr. Osborne O. Heard, Senior Modeler, who was appointed to the technical staff in 1914 as one of the first to join the Department, retired on March 20, 1956. Mr. Heard's term of service covered almost the entire history of the laboratory. He brought to his tasks not only superb technical ability but scientific accuracy and sound judgment. Although he described many of his technical innovations in scientific journals, his major contributions to the laboratory often were unheralded, but not unappreciated by his co-workers.

Mrs. Mary McCarthy Cope, Assistant Illustrator and Modeler, resigned on February 15, 1956, after ten years of service to the Department. Her friendly and unselfish assistance has been appreciated by all those who have worked with her.

But what of the future? What are the guideposts for the orientation of research, for the selection of new staff members? Our aims for the future do not differ greatly from those of earlier years when stated in general terms. The Department may be likened in one sense to an organism or to a large protein molecule. Although there has been considerable change or turnover within its parts, as a whole it enjoys stability. We achieve what Rudolph Schoenheimer liked to call a condition of *stability with flux*. Our object is to work toward a better understanding of the mechanisms of development, using for that purpose whatever methods become necessary. Our primary attention will be devoted to study of the vertebrate embryo, including the embryology of man and of as many species as is desirable. Yet we shall extend our interests to other developmental systems, for example regeneration,

morphogenesis in the invertebrates, or differentiation in microorganisms, whenever it seems profitable to do so. The great strength of the Department has been the association and interplay of morphology and physiology. Research in the coming years will, no doubt, call more heavily upon the methods of immunochemistry, physical chemistry of proteins, radiobiology, and fine-structure analysis. Physical facilities are being strengthened to that end. Yet there is a tendency for all biology today to be rushing headlong down the narrow path labeled nucleic acid and protein synthesis. Perhaps the most difficult task for the future will be that of maintaining the high quality of research in morphology and experimental morphogenesis, fields which are today less stylish than formerly. But strength must be maintained in these areas, for, inevitably, studies of the synthesis and interaction of specific molecules must be correlated with our knowledge of interactions at the cell and tissue level. It is at this level of analysis that students of development find their greatest challenge.

It seems desirable to defer a detailed account of the projected research program of the Department for a future report, when the plans have been implemented and the lines more clearly drawn, and to restrict comment at this time to a brief statement concerning the appointment of new investigators.

Dr. Royal F. Ruth, Associate in Embryology at Indiana University, was appointed to the staff on June 1, 1956. He is expected to conduct investigations in the field of immuno-embryology using the techniques of protein chemistry and cytochemistry.

Dr. Robert L. DeHaan, a recent graduate in embryology and physiology at the University of California at Los Angeles, joined the group, effective July 1, 1956. He plans to carry on studies in the biochemistry and physiology of development, emphasizing problems in cardiogenesis.

Dr. Malcolm S. Steinberg, a recent graduate of the University of Minnesota in the field of experimental embryology,

has been appointed a Fellow of the Carnegie Institution of Washington and will assume this position on September 1, 1956. Dr. Steinberg, who has conducted investigations in problems of coelenterate and amphibian development, will center his attention on the field of embryonic induction.

In addition, during the coming years the liaison between the Department of Embryology and the Department of Biology at the Johns Hopkins University will be strengthened by the establishment of a joint program in experimental embryology for a selected group of graduate students. The initial appointees in this program are Robert G. Beard, Fred Wilt, and Charles Wyttenbach, all of whom are transferring their activities to Baltimore from Indiana University.

In keeping with past tradition, during the year covered by this report the members of the Department have enjoyed the collaboration of a number of fellows and visiting investigators.

Dr. Vincent J. De Feo joined the Department on July 1, 1955, as a Fellow of the United States Public Health Service. In association with Dr. Reynolds, he has been engaged in a preliminary study of the pharmacologic inhibition of ovulation in monkeys by reserpine; in addition, Dr. De Feo initiated an experimental study of deciduoma formation in the rat.

Dr. Gilbert S. Greenwald continued research on the role of endocrinological factors in early development, during the second year of tenure of a fellowship awarded by the United States Public Health Service.

In collaboration with Dr. David W. Bishop, Dr. Seymour Katsh is engaged in an analysis of the differentiation and growth of the embryonic and adult gonad as influenced by homologous organ extracts. This investigation is conducted under the auspices of the Population Council.

During the summer of 1955, Dr. Csapo had the active co-operation of two Fellows



of the Carnegie Institution of Washington, Dr. Mavis Joan Middlebrook and Dr. Douglas Wilkie, who served with him at the Marine Biological Laboratory at Woods Hole, Massachusetts.

Dr. Chester H. Heuser, Research Associate, returned to the laboratory for several months in the summer of 1955 to continue work on the early stages of the human embryo (stages i to x, which were not included in George L. Streeter's *Develop-*

*mental Horizons in Human Embryos*). Other workers who have visited the laboratory in connection with investigations in progress include: Dr. Carl G. Hartman, former Staff Member, who carried on a study of tubal insufflation in the rabbit in collaboration with Dr. Reynolds; Dr. J. F. Albright; Dr. Roy E. Crowder; Dr. John D. Des Prez; Dr. Perry W. Gilbert; Dr. A. M. Mun; Dr. N. W. Vogel; and Dr. Lawrence R. Wharton, Jr.

## THE EMBRYO AND ITS ENVIRONMENT

### TRANSPORT, ATTACHMENT, AND MAINTENANCE OF THE EARLY MAMMALIAN EMBRYO

During the past year, an important part of the research effort of the Department has been directed toward a better understanding of the critical period in mammalian development in which the embryo is transported to the uterus, becomes implanted, and begins its intrauterine career. Although, as will be clear throughout this report, a number of different experimental approaches to this problem are being followed by members of the group, the investigations of Dr. Böving and Dr. Greenwald should be cited specifically at this point. Their studies have been carried on independently, but with a continuous valuable exchange of criticism and information.

It is curious that the blastocysts of the rabbit become almost evenly spaced along the length of its uterus. It is still more curious that this phenomenon occurs regardless of the number of blastocysts in a uterine horn; the implication is that the uterus can somehow "count" them and "divide" itself accordingly. Moreover, it is reasonably certain that the spacing is accomplished by uterine muscle without significant nervous control. During the past year, Dr. Böving completed and published a statistical analysis of the location of blastocysts in rabbit uteri on successive days. This investigation not only provided the first quantitative general description of blastocyst transport and spacing from the

time the embryos enter the uterus until they attach to it, but also permitted an accurate prediction of the normal location of every blastocyst in a uterine horn. Thus, it provided a standard for evaluating the effect of various experimental procedures on blastocyst transport and spacing. From 3 to 5 days post coitum the eggs were transported most rapidly but were arranged at random, whereas from 5 to 7 days post coitum the transport became slower and the eggs gradually became evenly spaced. The transition from random to even spacing was found to occur simultaneously with a rapid increase in egg diameter and certain changes in uterine muscle behavior. From the work of Corner, Csapo, and others, both the expansion and the special muscle behavior were known to be dependent on progesterone. These facts suggested that spacing was accomplished by interaction of the two progesterone-dependent mechanisms. The role of progesterone domination of uterine muscle was established by Dr. Greenwald's observation that amounts of estrogen similar to those known to interfere with progesterone-dominated muscle action did, in fact, cause abnormal spacing if given shortly before the time when even spacing usually develops. The role of blastocyst diameter in spacing has been explored by inserting glass or paraffin spheres of certain size into the empty horn of unilaterally pregnant rabbits. Results so far have been generally affirmative but not conclusive, and further work is planned.



Previous studies of rabbit blastocyst attachment to the uterus had revealed that a noncellular envelope or membrane provides adhesion, and that this noncellular membrane around the blastocyst increases in volume after the blastocyst enters the uterus. This suggested that the uterus may provide the second portion of the membrane, an idea that was confirmed by the histological observation of such a membrane deposited around paraffin and cork spheres which had been inserted into uteri of pregnant rabbits.

Complementing these findings are the results obtained by Dr. Greenwald, whose point of departure has been an analysis of the mechanism of interruption of implantation and maintenance of the rabbit blastocyst by injected estrogen. It was reported, in confirmation of results of preliminary experiments by Dr. Csapo, that as little as 5 gamma of estradiol daily for 3 days, commencing on the second day after fertilization, will interrupt pregnancy in the rabbit. The injected estrogen prevents the release of mucin from the tubal epithelium; consequently the tubal eggs are coated with only a thin "albumen" layer. These eggs develop normally in the tubes, but on entering the uterus at 3 days post coitum the blastocysts fail to undergo their characteristic expansion, and pregnancy is, therefore, terminated. These observations have been verified during the past year, and the research has been extended to other aspects of the problem.

Using castrated rabbits, it has been shown that estrogen is involved ordinarily in the synthesis and storage of mucin, whereas progesterone functions in the release of mucin from the tubal epithelium. Estrogen given to the rabbit after mating antagonizes the normal effect of progesterone. When progesterone is administered to rabbits during the first 72 hours after fertilization, the eggs are covered with twice as much mucin by volume as is found on untreated eggs. Conversely, when estrogen is given before mating, the mucin layer is reduced to traces. Mucin is deposited on

glass beads inserted into the tubes of ovariectomized rabbits treated with estradiol followed by progesterone, but not after estrogen alone. Thus, an excellent procedure for detailed chemical analysis of uncontaminated secretion is available.

The insertion of as little as 0.25 gamma of estradiol in 0.125 ml of corn oil into the right tube of rabbits significantly reduces the volume of deposited mucin, while the control tube contains eggs with normal amounts of mucin. Animals treated in this manner usually have unilateral pregnancies at 8 days post coitum, restricted to the untreated side of the reproductive tract. Preliminary experiments suggest that embryonic death in these animals is a consequence of increased tubal motility, in that the eggs enter the uterus earlier than they would under normal conditions.

#### THE HEART-FORMING AREAS OF THE EARLY CHICK EMBRYO

The cellular processes occurring during embryonic development, the period in which the fundamental character and specificity of the tissues composing the organism are established, present a series of challenging problems. Whereas in the adult, under normal conditions, the cells produce molecular species essentially similar to those already present, during embryogenesis and in other developmental systems, e.g. in regeneration, new types of synthesis must come into operation. It has been argued that chemical kinetic theory provides several different models for systems of interacting synthetic processes which will change progressively toward one or another of a number of alternative steady states, the direction depending on the initial conditions. It is during early development that the demonstration of distinct alternative pathways of chemical change, leading to the establishment of a finite number of tissues, is most critical. During the past year, Dr. Ebert has continued to explore the chemodifferentiation of the

heart-forming areas of the early chick embryo. In collaboration with Lowell M. Duffey (Indiana University), he completed the initial phases of an analysis of the metabolism of the cardiogenic tissues. The findings of this study were presented at the annual meeting of the American Association of Anatomists, at a special session honoring Professor B. H. Willier. By way of background, it will be recalled that the pattern of synthesis of the cardiac contractile proteins, actin and myosin, was established in experiments employing immunochemical techniques. It has been shown that one of these specific proteins, cardiac myosin, is synthesized throughout the epiblast of the early embryo, and then, as regional differentiation is initiated, disappears, or at least becomes undetectable, in all parts except the actual rudiment of the organ to which it belongs, the heart. As Waddington has pointed out, it might perhaps be that its disappearance is illusory because it fails, outside the heart-forming areas, to increase as fast as it does in them, or as fast as other substances do. But the evidence suggests that this is improbable; it seems far more likely that the disappearance is real. Clearly, these findings are compatible with the hypothesis that in development we have to deal with systems of competing processes. It is interesting to note that recently Langman has presented evidence of a similar loss of specific lens proteins during the development of the lens.

In the experiments now completed, distinct metabolic properties of an organ-forming area, namely the heart-forming regions, have been demonstrated for the first time. The initial objective of the study was to analyze the inhibition of cardiogenesis in the early embryo by sodium fluoride (reported by Spratt, 1950, and confirmed fully except for minor differences in effective concentrations). In addition to sodium fluoride, antimycin A has been shown to be, in the effective concentration range (0.02 to 0.1 microgram per embryo), a specific inhibitor of heart formation. These

specific effects have been contrasted with the general inhibition of development by other inhibitors, including amino acid analogues. Evidence has been obtained, from a study of over 750 explanted embryos, which demonstrates that the heart-forming areas are differentiated metabolically as early as stage 4 (Hamburger and Hamilton series). The most striking finding concerns the effect of sodium fluoride on the embryo, when analyzed at short intervals after explantation. Although individual differences, such as stage at explantation and energy reserves, cause variation, embryos cultured in  $10^{-2}$  M sodium fluoride for 2 to 5 hours show marked areas of degeneration which correspond in position at stage 5 to the localization of cardiac actin and myosin, and to the heart-forming areas as revealed by transplantation techniques. At stages 6, 7, and 8, the position of the degenerating regions reflects the anteromedial movements of the heart-forming cells. Embryos cultivated for brief periods in a medium containing the inhibitor, marked with fine carbon particles at the initial sites of inhibition, and subcultured in a normal medium lacking sodium fluoride, recover and develop normally. Invariably the carbon particles are located in the heart, a fact which supports the argument that the zones of inhibition are indeed the heart-forming areas.

These provocative findings pose a series of further questions, among which are the following: In the regions affected by the inhibitor, is the synthesis of the cardiac proteins blocked, or is the effect at the level of morphogenesis, on the fashioning of the contractile elements into their characteristic structure? Is there an alteration in the pattern of differentiation of the sarcosomes? It is suggested that the chemical site of inhibition is at the level of the mitochondrial adenosine triphosphatases. What are the relations between mitochondrial differentiation and the course of muscle fibrogenesis? In order to prepare for the task of unraveling these questions, it has been necessary to work out methods

for the quantitative estimation of cardiac myosin, which serves as an excellent example of those tissue proteins that present difficulties from the standpoint of solubility properties (R. F. Ruth), for the determination of organ and species specificities of sarcosomes (J. F. Albright), and for elucidating, at least in part, the factors involved in immune cytotoxic reactions (A. M. Mun). These studies, which have been useful beyond their original purpose, now can be brought to bear on the specific problems cited in the foregoing discussion, and on other problems of development.

#### DEVELOPMENTAL HORIZONS IN HUMAN EMBRYOS

During the year, Dr. Chester H. Heuser and Dr. George W. Corner completed for publication in volume 36 of the Contributions to Embryology a full description of age group x (4 to 12 somites) in the normal development of the human embryo. The investigations dealt with 11 human embryos in the Carnegie Collection; the estimated ovulation age is  $22 \pm 1$  days. In addition, brief evaluation is made of the salient features of 27 additional embryos usefully cited in the literature. In specimens of horizon x, the fusion of the neural folds is imminent or in progress, the optic sulcus is present, the mandibular and hyoid bars can be seen externally, and from 4 to 12 pairs of somites are present in embryos ranging in length from 1.5 to 2.0 mm. Attention was paid to the development of the heart and nephric system. In the youngest members of the group, the endocardial rudiment is plexiform, whereas the oldest embryos show a definite S-curve of the bulbus cordis and ventricular endothelial tube. Nephric structures first appear in horizon x; primitive segment stalks with nephrocoels and peritoneal funnels are present in 7- to 8-somite embryos; other parts of the nephrons develop later.

#### HISTOGENESIS AND ORGANOGENESIS

*Adrenal Gland.* Dr. Roy E. Crowder, Captain, Medical Corps, U. S. Navy, con-

cluded his analysis of the development of the adrenal gland of man. His findings and conclusions are being assembled for publication in volume 36 of the Contributions to Embryology. On the basis of a cytological study of the adrenal gland throughout ontogeny, Dr. Crowder challenges the concept of the "fetal cortex"; he argues that the capsule and glomerular zone are the germinal regions for the entire cortex. As a result of his analysis of the origin and ultimate location of the several types of cortical cells, and after consideration of the limited experimental evidence available, he emphasizes that the "cell migration" theory cannot be rejected. Clearly, the apparently conflicting points of view must be resolved by experiment; it would appear that the methods are at hand; recent successes in the culture of intact embryonic organs suggest that a study of cytodifferentiation in explanted whole and enucleated adrenal glands might be rewarding. One might ask also concerning the developmental capacities of lines of cells isolated from the glomerular zone.

*Chondrification in Hands and Feet.* Dr. Ronan O'Rahilly and Dr. Ernest Gardner, of the Department of Anatomy of Wayne University, and Dr. D. J. Gray, of the Department of Anatomy of Stanford University, completed during 1955 several parts of a large program of studies of the development of the human hand and foot. One aspect of the investigation, dealing with chondrification in the hands and feet of staged human embryos, will be published in the forthcoming volume of the Contributions to Embryology. The group studied the early development of the limb in serial sections of 103 human embryos, ranging from Streeter's horizon xv to xxii inclusive (approximately 6 to 32 mm in length), and have provided a detailed account of the order of appearance of the skeletal elements and the sequence of chondrification in the hand and foot.

*Development of the Cranial Venous System.* Mrs. Dorcas H. Padget, former Fellow, completed for publication in the Con-



tributions to Embryology an extensive and fully illustrated description of the development of the venous system of the head in human embryos, for which there has been a need for a number of years. Mrs. Padget's material encompassed, in addition to the resources of the Carnegie Collection of embryos, dissections of the heads of a large number of fetal, newborn, and adult humans, and a considerable body of material for comparative anatomical study, including especially prepared heads of the cat, dog, and rhesus monkey. Her findings have shown in general that both the ontogenetic and phylogenetic patterns of development of the cranial vasculature must be visualized in order to prevent misconceptions concerning its subdivisions in embryonic or mature specimens. Attention is called also to a companion article in the *American Journal of Anatomy*, which treats several more obscure features of the pattern of the cranial vasculature in man from the developmental point of view. This article includes a discussion of embryologic factors involved in the origin of congenital arteriovenous aneurisms.

*The Occipital and Cervical Segments.* The segmentation of the occipital region has been of interest to embryologists since Goethe advanced the vertebral theory of the origin of the skull. The hypothesis of a vertebral origin of the entire skull served a useful purpose as the forerunner of a number of studies which have shown that at least the postotic portion of the cranium is derived from segmented primordia, the occipital somites. Dr. E. Carl Sensenig, of the University of Alabama Medical Center, has completed an intensive re-examination of the development of the postotic portion of the cranium and of the upper cervical segments in man, using the embryos of the Carnegie Collection. Dr. Sensenig concludes that at least four, and possibly five, occipital segments are incorporated in the human occipital bone. The contribution of the fifth, most rostral, segment cannot be established without the application of experimental methods. In addition, he has

presented an account, more detailed than has been available previously, of the later development of the occipitocervical region, including a study of the formation of the atlas and axis. Dr. Sensenig's paper will appear as number 248 in the Contributions to Embryology.

*Ocular Muscles.* Dr. Perry W. Gilbert spent the period September 1, 1949, to January 31, 1950, in the Department as a Fellow of the Carnegie Institution while on sabbatical leave from Cornell University, and has been a frequent visitor to the Department since that time. His remarkable observations on the origin and development of the head cavities in the human embryo were summarized in Year Book No. 50 (1950-1951) and published in the *Journal of Morphology* in 1952. Now Dr. Gilbert has prepared a comprehensive report on the development of the human eye muscles. His convincing demonstration that in man the extrinsic eye muscles arise from three independent but closely apposed mesenchymal condensations situated at each side of the head, beautifully illustrated by the late James F. Didusch and Mrs. Mary McCarthy Cope, will appear in volume 36 of the Contributions to Embryology. The origin and development of the eye muscles in man follows a pattern that conforms in essential features with that found in the cat, marsupials, birds, reptiles, and elasmobranchs. The widespread concept that the extrinsic eye muscles of man arise from a single primordium on each side of the head should be abandoned.

*Temporomandibular Joint.* The contents of Dr. Benjamin C. Moffett's article on this subject, which is scheduled to appear in volume 36 of the Contributions to Embryology, were summarized in Year Book No. 54 (1954-1955).

*Trachea and Esophagus.* As reported in Year Book No. 49 (1949-1950), Dr. Edwin Ide Smith, Halsted Fellow in Surgery at the Johns Hopkins University, spent the year July 1, 1949, to June 30, 1950, in the Department of Embryology. He made a comprehensive study of the embryogenesis



of the human thorax, and more particularly of the trachea and esophagus. His publication on that subject, which will appear in the Contributions to Embryology, deals specifically with the development of the trachea and esophagus in early embryos, horizons x to xv as defined by Streeter. Fifty-eight embryos in this period, approximately 21 to 32 days of development, were analyzed. In addition, Dr. Smith takes up the origin of several congenital defects, especially atresia of the esophagus and tracheoesophageal fistula, in the light of his observations on normal development of these tissues.

*A Table of Human Development* was designed and prepared by Dr. Böving for the *Handbook of Biological Data* being assembled under the auspices of the National Research Council. In an effort to provide up-to-date and reliable information, the data were obtained primarily from consultants expert in particular organ systems. These data were supplemented, where necessary, by data from generally accessible and well regarded publications, including textbooks. They were then reduced to symbols and tabulated (according to anatomical system, structure, and subdivision) on a logarithmic time scale to which were related all the various time and anatomical scales commonly used in embryology. This procedure brought into relationship data originally given in unrelated units, and permitted data to be interpreted in whatever units might be desired. The table not only provides a condensed history of the development of each structure, but, since all are tabulated on the same time scale, also permits correlation of the development of any one structure with that of any other. In addition, one may read "across" all structures at any stage and obtain a rather complete description of the organism at that level of development. Conversely, the best fit between such a description and the characteristics of a specimen of unknown age may be used to estimate its age. Since human development does not stop at the time of birth, the table,

in contradistinction to most embryological accounts, was extended to cover the full life span.

#### SEX DIFFERENTIATION

In many species of amphibia and in the chick embryo, the administration of sex hormones during the period of sexual differentiation may lead to a transformation of the gonad by reversing the normal, genetically determined balance between the cortical and medullary components. The development of the accessory sex structures can, of course, be altered readily. In the mammal, however, even in cases in which the accessory structures have been transformed strongly, there has been an apparent failure of sex hormones to induce significant histological changes in the gonads. Thus, the stand of those who argued that the steroid sex hormones of the adult are probably not similar to the sex-differentiating substances of the embryonic gonad was supported at least in so far as negative findings are capable of lending support to any argument. On the other hand, it has been clear that during its development the mammalian gonad possesses the requisite structural basis for sex transformation. It seemed likely, therefore, that the previous conspicuous failures reflected only a failure in experimental design or timing. As reported briefly in Year Books Nos. 53 and 54, and published during the past in the *Proceedings of the National Academy of Sciences*, Dr. Robert K. Burns has demonstrated convincingly that in the opossum the earlier negative findings can be attributed to a failure to administer the steroid hormones at a sufficiently early point in development. In a number of cases, when estradiol dipropionate was administered to genetic males, the transformed gonads closely resembled ovaries. The success of the experiments hinges on the observation that there is considerable variability in the stage at which viable embryos are born; thus, it is possible to select litters which are born earlier than usual, and to treat them at the earliest possible

moment after birth. In three experimental litters that received as little as 0.15 microgram of the hormone daily, the internal organs of sex were all strongly transformed, and the copulatory organs, of female type, were hypertrophied. The structure of the gonads approaches that of ovaries very closely. Not only is the cortex well developed, but germ cells are also present, although in reduced numbers. Certain peculiarities, however, remain to indicate the testicular origin of the gonads. These striking results must be extended; questions such as the permanence of the transformations, the later history of the germ cells, and the fate of the surviving medullary structures demand attention.

In a paper published in *The Corner Festschrift Volume*, Dr. Burns has extended his earlier analysis of the mechanisms by which sex hormones influence the growth of the homologous organs. It had been observed, for example, that in young opossums treated with sex hormones during sexual differentiation, with identical dosages of male hormone male sex structures consistently attain a larger size in males than do the homologous structures in littermate females. Conversely, identical dosages of female hormones always lead to greater development of female sex structures in female subjects than in males. It was concluded originally that the observed growth differences are based on physiological differences in receptivity or capacity for growth, which are inherent in the individual sex primordia by virtue of sex constitution. This conclusion is supported by new evidence. First, evidence from collateral sources has demonstrated that whereas in mammalian embryos the interstitial cells of the testes produce a hormone essential for normal development of the male structures, there is no evidence that the ovary produces a hormone during embryogenesis. Nevertheless, size differences of the same order, in favor of female sex structure, are found in female subjects receiving female hormone. Therefore, the possibility that the effects of

exogenous hormones must be enhanced by an endogenous substance is rendered unlikely. In addition, two new types of data are cited: (1) histological study of the testes of experimental animals lends no support to the view that they are active in an endocrinological sense, and (2) determinations of volume of a number of sex structures in experimental animals show that the difference in size between homologous structures in the two sexes is much too large to be accounted for on the basis of gonad activity.

During his stay in Paris, Dr. Burns prepared two additional papers for publication: "Vie et reproduction de l'Opossum américain," to appear in the *Bulletin zoologique*, and a further description of the transformation of testis to ovary under the influence of female hormones in the opossum, which will be published in *Archives d'anatomie microscopique et de morphologie expérimentale*. Their content will be reviewed in a future report.

#### ORGAN-SPECIFIC CONTROL OF DIFFERENTIATION AND GROWTH

The existence of a cell-type-specific chemical mechanism for correlating growth processes among homologous cell types may be regarded as demonstrated. Several lines of evidence have established the general idea of selective chemical communication among cells of identical types by direct exchange of protoplasmic type-specific compounds. Analysis of the mitotic index in embryonic and adult organs after administration of homologous triturated cell masses, either fresh or frozen and thawed (Weiss, Andres, Teir), the increase in the undamaged liver of one partner of a pair of parabiosed rats following partial hepatectomy of the other partner (Bucher, Scott, and Aub), the increase in rate of liver regeneration when the blood is diluted (Glinos and Gey), and the selective incorporation in embryonic organs of radioactively labeled molecules from grafts of homologous organs (Ebert, Waddington) support the tentative, and probably over-

simplified, hypothesis that the co-ordination and regulation of growth processes presupposes the generation in each cell strain of paired compounds of complementary configuration. Whereas the methods employed to date have been adequate to demonstrate the specificity of the phenomenon, they have not proved to be either appropriate or sufficiently sensitive to reveal the nature of the mechanisms involved. For example, it has not been established fully whether the effect of an organ brei or graft must be mediated by intact cells or whether subcellular or molecular inocula can be effective. Moreover, there is no clear-cut evidence to determine whether the effective agents act directly on the homologous tissue or whether the effect is mediated by an immune response. The latter possibility is usually discounted on the basis of the embryo's inability to produce circulating antibodies; the question of a cellular immune response has not been examined. Comparison of the findings on growth stimulation in normal tissues with the literature bearing on the tumor enhancement reaction clearly suggests that experiments should be designed to detect mediation of the effects of organ grafts by an immune mechanism.

Another question that urgently demands attention springs from the reports suggesting that differentiation and growth of a tissue may be affected in opposite ways by administration of the homologous material. It is fair to state that there has been no critical or complete analysis of this possibility for any one tissue. One of the more interesting descriptions of the effect of a tissue extract in inhibiting differentiation is the report by Freund that water-in-oil emulsions of guinea pig testis block spermatogenesis in the guinea pig. This observation raises a number of questions which are exciting to the embryologist and immunochemist alike. In consultation with Dr. David W. Bishop, Dr. Seymour Katsh has undertaken a broad program of research in this direction. His investigation, which has been under way for only a short

time, is concerned with attempts to alter the structure or function of the male and female gonads and of the accessory organs of reproduction, and to influence the development of embryos in utero by gonadal extracts. Questions of organ and species specificity are being examined in rats, rabbits, guinea pigs, and chickens.

To date, progress has been made in altering the male gonad of the guinea pig: The testes of guinea pigs, which receive intradermal injections of a water-in-oil emulsion of guinea pig testes and bacto-adjuvant (paraffin oil, Arlacel oil, and dead mycobacteria), or of spermatozoa of several species, are found to be almost completely devoid of spermatogenic elements 6 to 8 weeks after initiation of the injections. High antibody titers are also found in the sera of these animals.

#### PHYSIOLOGY OF THE FETUS

*Fetal Circulation.* During the year covered by this report, the team of S. R. M. Reynolds and William M. Paul (R. Samuel McLaughlin Foundation Fellow) published the results of experiments, described in Year Book No. 54, designed to measure and analyze the effect of intrauterine pressure on blood pressure and heart rate in the fetal lamb. The authors develop the hypothesis that fetal bradycardia upon increase of uterine pressure is the result of acute hypoxia of the fetus, associated with interference with the fetal circulation. A number of experiments, e.g. the application of pressure to the uterus by the addition of weights or the injection of Pitocin leading to uterine contraction, strongly suggest that the bradycardias commonly associated with increased uterine pressure result from central anoxia that leads to inhibition through the vagus nerve.

In Year Book No. 54, Dr. George W. Corner stressed the importance of a continuous exchange between the fields of morphology and physiology. Two publications by Dr. Reynolds and his co-workers illustrate strikingly the value of such interplay. Vittorio L. Danesino, S. R. M. Reyn-



olds, and I. H. Rehman have described the histological structure of the ductus arteriosus in man with reference to differences in histo-architecture throughout its length, and to age and degree of constriction. Their aim is, of course, to correlate the structural characteristics of the ductus arteriosus with hemodynamic conditions that undergo adaptive alterations at the moment of birth and with the initiation of pulmonary ventilation. Differences in arrangement and quantity of muscle fibers in the cephalic and caudal portions of the ductus arteriosus are described. In addition, it is noted that the presence of sub-endothelial cushions (which are absent from the ductus arteriosus of young fetuses) indicates a constricted and shortened ductus arteriosus. It is argued, therefore, that the ductus arteriosus is less constricted in younger fetuses than it is in older specimens.

In another publication, Dr. Reynolds has considered the fetal and neonatal pulmonary vasculature in the guinea pig in relation to hemodynamic changes at birth. The hemodynamic basis of the study lies in the following observations: pulmonary arterial pressure is high in the fetus but low after birth; blood flow is slow before birth but fast and voluminous after birth; pulmonary peripheral vascular resistance is high in the fetus but low after birth; pulmonary blood volume is smaller in the fetus than it is after birth. On the basis of study of the fine vascular architecture of the lung of fetal, newborn, one-day-old, and adult guinea pigs, he concludes that in the fetus resistance to blood flow in the lung is high owing to the presence of coiled and erythrocyte-packed arterial capillaries and to the high left atrial pressure from the flow of blood through the foramen ovale. On aeration of the lungs, the extension of the arterial capillaries in the lung lowers the resistance to blood flow and permits extension of a previously closed capillary plexus with consequent increase in pulmonary blood volume.

The clinical implications of the hemo-

dynamic character of the fetal circulation and the circulatory changes that occur at birth are treated in a paper by Dr. Reynolds which appears in the *American Journal of Obstetrics and Gynecology*.

#### PHYSIOLOGY OF THE OVARY AND OVIDUCT

*The Ovary.* Few students of reproductive physiology have considered the relation of the ovarian vasculature to ovarian function. In recent years, Dr. Reynolds has emphasized the importance of proper hemodynamic conditions in the ovary, an organ which, on the one hand, is concerned with the periodic production of ova, and, on the other hand, produces the hormones estrogen and progesterone. During the year, Dr. Reynolds brought together many of his own observations and the findings of others on the control of ovarian blood pressure and the distribution of blood within the ovary in a review article dealing with "The Ovarian Vasculature and Ovarian Functions." The interdependence of the ovarian stroma and its vasculature is made clear, inasmuch as the development of the ovarian vasculature depends largely on the production of estrogen by the ovary itself.

*The Tubal Fluid.* During the past year, Dr. Bishop completed the initial phases of several projects that bear on the nature of the tubal fluid of the rabbit—factors which control its active secretion, its biochemical composition, and its significance in relation to the metabolism of spermatozoa and blastocysts. It should be noted first that determination of the secretory pressure of the tubal epithelium has proved to be a rough bio-assay of the estrogen level. By a combination of methods involving catheterization and manometric recording of ligated oviducts of anesthetized animals, the rate of tubal secretion and the maximum secretion pressure attained have been found to be high in estrous rabbits and low in progestational animals, and to decrease with the duration of the stage of pregnancy. Castration abruptly reduces secretory activity, and estrogen adminis-



tration promptly restores it. The estrous level of secretion pressure, 40 to 55 cm of water, corresponds to the lower range of arteriolar blood pressure in the oviduct. Tubal secretion is pilocarpine-sensitive.

Biochemical analyses of tubal fluid, with particular emphasis on potential metabolic substrates, have revealed the absence of glucose and fructose but the presence of significant quantities of lactate and phospholipid. Electrochemical determinations by means of an "oxygen microcathode" demonstrated an aerobic environment in the interior of the oviduct; the oxygen tension of the tubes of estrous rabbits ranges from 30 to 55 cm of mercury, thus indicating equilibrium with blood oxygen concentrations. The data have been interpreted as evidence against compulsory anaerobic glycolysis of sperm and eggs—a theory held by various investigators in the field of reproductive physiology—and evidence for the oxidative respiration of the reproductive cells. In the absence of available tubal substrates, the sperm utilize endogenous phospholipid reserves; this, in the final analysis, is believed to be one of the deciding factors in the survival of sperm and in their ability to fertilize eggs.

The detailed findings of these studies will be available shortly in several papers now in press and in preparation.

*Tubal Insufflation in the Rabbit.* During the year, Dr. Reynolds completed the installation and calibration of equipment and standardization of techniques for cinefluorographic recording of body activities. Progress in the cinefluorographic program had been halted by delay in delivery of the synchronizing unit, which was essential for photography of the fluorescent screen. With its arrival, work on a number of problems was begun, and experience was gained in photographically recording in slow motion a number of physiological activities, including distention coefficients of the aorta in situ, and the regional redistribution of blood after injection of adrenaline.

The principal accomplishment was a

study of the mechanism of tubal insufflation in the rabbit, carried out in collaboration with Dr. Carl G. Hartman, formerly of the Department, now Associate Director of the Ortho Research Foundation. The study involved X-ray visualization of the Fallopian tube at the beginning of perfusion and as perfusion was continued under controlled pressure. The rate of flow was recorded on the X-ray motion picture as drops of fluid (opaque medium) were passed through the tube, and records of the pressure were obtained.

Two opposing views have been expressed regarding the mechanism of resistance to passage of fluid through the tube: first, that there is a sphincter-like mechanism at the uterotubal junction; and second, that the resistance is solely the result of rhythmic activity of the uterus about the tubal insertion. In the present work, the entire tube was seen and photographed cinefluorographically. It was studied under three hormonal conditions: in the ovariectomized rabbit, in the rabbit treated with estrogen, and in the rabbit treated with progesterone. In brief, it was observed that in the estrogen-treated rabbit a high initial pressure is required to force the fluid through the entire isthmus of the tube; once the fluid is flowing, the isthmus intermittently contracts and relaxes in segments. The flow alternates between little or none, and quite rapid movement. In castrated and progesterone-treated rabbits, the high initial resistance to perfusion is not encountered, and the segmental rhythmic activity is less strong. Though further studies need to be made, it is clear that the chief resistance to tubal perfusion lies in the segmental activity of the musculature of the entire isthmus.

*Sperm Transport in the Reproductive Tract of the Female Rabbit.* In connection with Dr. Bishop's analysis of the tubal fluid, with special reference to the physiology of sperm, brief mention may be made of a series of experiments completed recently by Dr. Greenwald. Previous estimates of the time required for sperm

ascent in the reproductive tract of the female rabbit have been obtained by flushing the tract at definite intervals after mating. Another approach to the problem has been devised, namely ligating the tubes at the uterotubal junction at various times post coitum and then examining the trapped eggs for evidence of fertilization after 48 to 52 hours. The results indicate that sperm enter the tubes from 2 to 5 hours after mating.

#### PHYSIOLOGY OF THE UTERUS

##### *The Myometrium*

In *The Corner Festschrift Volume* of the *American Journal of Anatomy*, Dr. Arpad Csapo has summarized the current state of knowledge concerning progesterone "block," i.e. the effect of progesterone in preventing the maximal contraction of the myometrium. His article is singularly appropriate to the volume, for it was the classic observation by Allen and Corner which demonstrated by replacement therapy that a hormone of the corpus luteum is responsible for progestational changes of the uterus and is prerequisite to normal gestation. One is justified in saying that a number of observations provide conclusive evidence that, in the rabbit at least, progesterone plays an indispensable role in the maintenance of pregnancy. The understanding of the progesterone effect is another matter. It is complicated by apparent species differences in the significance of the corpus luteum in the maintenance of pregnancy, by a possible shift in the site of progesterone synthesis during gestation, by the inconclusiveness of the results of progesterone substitution therapy in the human in cases of imminent abortion and premature labor, and by lack of critical information concerning the mechanism of action of the steroid hormones. It is against this background that Corner and Csapo initiated a series of biochemical and physiological studies of the mechanism of action of progesterone. Their research was centered about the questions: At what

level of organization does progesterone affect the myometrium? What does it do? The first significant observation was the finding that the final contractile system (actomyosin-ATP) was not noticeably different under estrogen and progesterone domination. The action of progesterone is at a higher level of organization of the excitation-contraction process. It is suggested that the hormone upsets the ionic balance of the myometrial cell; it seems likely that the disturbance leading to increased sodium and decreased potassium concentrations inside the cell may be due to inefficiency of the "sodium pump" mechanism; alternative explanations, such as changes in membrane permeability or in the efficiency of carrier systems, must be considered.

It is reassuring to note that many of the conclusions advanced on the basis of studies of the uterus in vitro are supported by comparable investigations carried out in vivo. The work of Dr. Brenda M. Schofield serves as an excellent case in point. Experiments cited in Year Book No. 54 demonstrated that in the intact animal, as well as in isolated uterine strips, the form of the contraction wave, the relation between tension and strength of stimulus, and the direction of the staircase (the development of maximum tension by a series of stimuli at constant strength) all depend on the action of estrogen or progesterone. In an article published during the past year, Dr. Schofield has reported the findings of a continuation of these experiments. A statistical analysis of the tensions developed in uteri fully dominated by estrogen or progesterone indicates that the progesterone-dominated uterus develops less tension than the estrogen-dominated uterus when the tension is related to the weight or the length of the segment. Progesterone apparently produced no alteration in the character of spontaneous activity. These experiments also indicate that injected progesterone has a latent period: about 21 hours elapse between the injection and the beginning of change in char-

acter of the muscle contractility from the estrogen-dominated behavior.

During the past year, Dr. Csapo has been concerned largely with problems of threshold and spontaneous activity. The importance of studying the threshold in muscle lies in the fact that slight changes in threshold drastically alter the excitability and consequently the function of muscle. It had been established that in the myometrium, under well defined endocrine conditions, the threshold significantly changes from one endocrine state to another, and that this change in threshold explains to a great extent the functional behavior of the myometrium in any of the given endocrine states. Two parameters were found to have the largest effect on the threshold. One was the potassium ratio across the membrane, and the other, the calcium concentration in the perfusion bath. It was assumed that change in the calcium concentration in the perfusion bath affects the concentration of this same ion inside the cell. It was shown that in estrogen-dominated myometrium the threshold is a function of the logarithm of the potassium concentration outside. To determine the value of the potassium concentration outside at which threshold becomes zero is not possible by direct measurement, because with increasing concentration of potassium outside (decreasing membrane potential) spontaneous activity increases, and this increases the inaccuracy of quantitative measurements. But zero threshold has been obtained by extrapolation, and the critical concentration of potassium outside was found to be 30 mM. If the potassium concentration is further increased, the muscle goes into contraction. The effect of increased potassium in lowering the threshold is also shown by increased spontaneous activity. The first effect of increased potassium concentration outside (decreased membrane potential) is an increase in the frequency of spontaneous contractions. As the frequency increases, tension becomes greater, and at zero threshold the amplitude of spon-

taneous contraction is maximal, i.e. it is the same as that developed by the muscle when stimulated with an optimal electrical stimulus.

These studies led Dr. Csapo and Dr. Douglas Wilkie to study the dynamics of potassium action on the propagated response in frog muscle. They found, working on the frog sartorius, that in 12-mM potassium-Ringer the decay in action potential and in the normal tetanus response follows about the same time course, that is to say that both decrease together as the potassium enters the extracellular space. It was also found, however, that after the normal tetanus response fails, the muscle does not become electrically inexcitable. Increasing the strength of stimulation by some five times, one can attain partial tension development. Thus, under these conditions, it is possible to study the active state and the mechanical response in a non-propagating muscle.

It is not clear whether increased concentration of potassium outside produces a partial depolarization only, or whether, together with depolarization, a subsequent change also occurs inside the myofibrils.

The most significant finding in this series of experiments was the observation that when the muscle recovers from the effect of high potassium and is being repolarized, the recovery is greatly delayed in the cold (2° C), but instantaneous recovery occurs when the muscle is suddenly warmed up to room temperature. The strong temperature dependence of recovery indicates that metabolic processes are involved. Since radioactive isotope studies with potassium<sup>42</sup> and sodium<sup>24</sup> gave no satisfactory explanation for delayed recovery in the cold, the phenomenon cannot be explained by the effect of temperature on sodium and potassium fluxes.

*The Temperature Coefficient of Tetanic Tension.* There is considerable disagreement among muscle physiologists concerning the temperature coefficient of frog muscle. A detailed analysis of the question



revealed that the  $Q_{10}$  of the frog sartorius is 1.3.

### *Endometrium and Placenta*

*Endometrial Blood Vessels and Menstruation.* In Year Book No. 54, reference was made to a paper completed by Dr. George W. Bartelmez dealing with the ischemic phase of the reproductive cycle in the rhesus monkey and man. This paper, bearing the intriguing title "Premenstrual and Menstrual Ischemia and the Myth of Endometrial Arteriovenous Anastomoses," was published in *The Corner Festschrift Volume of the American Journal of Anatomy*. Dr. Bartelmez finds no evidence of the existence of the arteriovenous anastomoses of endometrial vessels which have been used by several investigators in constructing hypotheses of premenstrual ischemia.

In addition, Dr. Bartelmez has completed a paper on the vascular changes during the menstrual cycle in the rhesus monkey, with detailed diagrams of the changing pattern of the coiled arteries. The report, which will be published in volume 36 of the *Contributions to Embryology*, is based on the examination of doubly injected specimens. The observations are interpreted in the light of Markee's extensive observations on ocular transplants of endometrium and on Daron's intravital injections. Correlations are made with uterine reactions before fertilization and during early stages of pregnancy. The suggestion is made that the rate of blood flow determines the hormonal responsiveness of the various regions of the endometrium.

From time to time during the past twenty-five years, Dr. Bartelmez has presented evidence that the loss of tissue from the endometrium during menstruation both in the human and in the macaque does not involve three-fourths of the membrane as is usually taught. When adequate histologic methods are used on well preserved normal material it appears that the reduction in thickness is due largely to

the disappearance of ground substance from the stroma and to the reduction in size of the component cells as they dedifferentiate. The increased contortion of the coiled arteries with signs of compression is a striking feature of menstruation. Study of the uterine vasculature gives evidence that only the superficial rami of these arteries are lost during ovulatory menstruation. In any case, there are well differentiated arteries at the surface after menstrual sloughing. During the later follicular phase, the coiled part of these arteries is for the most part in the basal one-fourth. This has led to the belief that the superficial three-fourths of the endometrium is regenerated after every flow. The only evidence for this that has ever been presented is active mitosis in the epithelia. Mitoses are rare in the stroma, which constitutes the bulk of the tissue during repair and early follicular phases. They are not common in the coiled arteries until the gravid phase associated with an active corpus luteum. When adequate material is studied, it appears that the walls of the arteries, like other tissues, dedifferentiate; they uncoil as the ground substance again accumulates in the stroma, and later in the follicular phase they show signs of being stretched. Dr. Bartelmez also has under way a study of the series of changes in the arteries as they dedifferentiate and redifferentiate in the human and macaque. This investigation should also bring forth data on the structure of the endometrial veins and sinuses. Other work in progress includes a detailed description of the injection methods used for the uterus, which is completed except for a few illustrations. In addition, Dr. Bartelmez proposes to emphasize the inadequacy of the terms used exclusively in the medical literature for the subdivisions of the menstrual cycle. A preliminary statement of his views on this subject was made before the National Academy of Sciences in April 1955.

*Vascular Patterns in the Endometrium and Placenta.* During the past two years, Dr. Elizabeth M. Ramsey has participated



in the Annual Microcirculatory Conference, which brings together pathologists and physiologists interested in problems in the microcirculation in tissues, including factors regulating blood flow. Her contribution each year has been a consideration of the vasculature of the endometrium and placenta; the proceedings of the Conference are published in *Angiology*. Dr. Ramsey has set forth the argument that the intervillous space of the placenta is, in physiological effect, a small blood vessel, since it is the terminal area of metabolic exchange between the maternal and fetal blood streams. Her material embraces the rhesus monkey and man. The human uteri obtained at hysterectomy are perfused and injected with India ink. In the monkey, injections are carried out on animals bred and maintained for the desired period. Initially, India ink was injected by way of the aorta in anesthetized animals; more recently, colloidal mercuric sulfide, the dye originally employed by Bartelmez in studies of the vasculature of the nonpregnant endometrium, has been injected into the unanesthetized animal through the femoral vein. In these experiments, in which Dr. Ramsey frequently has had the effective co-operation of Dr. Bartelmez and Dr. Böving, the animals are tranquilized by preliminary administration of reserpine. Following the injection, the uterus and adnexa are fixed in situ to prevent myometrial contraction, and the material is prepared in thick sections to facilitate observation of the endometrial vascular network. It is important to note that recent improvements in experimental technique have yielded confirmation of the findings achieved with the earlier methods: maternal blood enters the intervillous space from the spiral arterioles under the head of maternal pressure, and proceeds toward the subchorial lake in distinct spurts. It does not "short-cut" into venous exits from the intervillous space even when they are contiguous to the arterial entry points. In addition, the independence of individual arterial units is demonstrated by the patchy

distribution of entering arterial spurts and venous drainage systems. Not all arteries contract and relax at the same time.

Dr. Ramsey has reviewed the information bearing on the distribution of arteries and veins in the mammalian placenta and has summarized the physiological concept of placental circulation in articles appearing in *The Corner Festschrift Volume* of the *American Journal of Anatomy* and in *Gestation, Transactions of the Second Conference*, of the Josiah Macy, Jr. Foundation. Her thesis embodies the following points: Distribution of arterial and venous channels along the base of the placenta is essentially indiscriminate; separation of afferent and efferent streams is effected primarily by pressure differentials in the intervillous space; morphological arrangements, e.g. placental septa, and the villous pulse do not effect the intervillous circulation, although they may promote mixing and assist it; myometrial contractions probably close the venous channels so that evacuation of the intervillous space is impossible, even though blood may be expressed from the uterine vessels into the systemic circulation.

These arguments, which are based on anatomical findings in macaque and human uteri, and on a review of the pertinent literature in the field of uterine physiology, provide convincing refutation of the older theories, e.g. that of Spanner, which recent experimental findings fail to confirm. In the course of her discussion, Dr. Ramsey questions the use of the terms "marginal sinus" and "maternal venous sinuses," and proposes that more accurately descriptive expressions, "maternal veins" and "marginal lakes," respectively, be substituted.

#### *Passage of Sugars across the Primate Placenta*

During Dr. A. St. G. Huggett's visit to the Department in 1954-1955, he and Dr. Reynolds led a team of investigators, including Francis P. Chinard, Vittorio L.

Danesino, William M. Paul, and William L. Hartmann, in a study of the transmission of hexoses across the placenta in the human and the rhesus monkey. In Year Book No. 54, Dr. Corner summarized the findings of the study with respect to the rhesus monkey. It has now been reported, in addition (see under Chinard *et al.* in the bibliography below), that no fundamental difference was detected between the monkey and the woman in regard to the passage of sugars from the mother to the fetus. The results cited in the previous report for the monkey, therefore, apply equally well for the human. The principal conclusions may be reviewed: The primate placenta does not form fructose from glucose when fetal hyperglycemia is produced; the normal primate fetal blood hexose appears to be glucose; glucose appears to pass freely across the primate placenta in both directions; fructose goes freely from the mother to the fetus when injected into the maternal circulation; there is a suggestion that an excess of glucose can impede the flow of fructose; radioactive glucose given to the fetus appears in the maternal blood with ease, but in the amniotic fluid only in small quantities.

#### *Clinical Studies*

Dr. Reynolds is a co-author of two reports resulting from collaborative studies in the fields of gynecology and obstetrics. W. M. Paul, B. I. Glickman, I. M. Cushner, and S. R. M. Reynolds completed their investigation of the pain thresholds of the human cervix uteri, intended to provide information concerning the nature of dysmenorrheal pain and a basis for evaluating pain-reducing drugs. Although, as indicated in Year Book No. 54, differences are noted in thresholds of painful response to distention between women who do and do not suffer from dysmenorrhea, psychosomatic factors affect uterine tonus markedly and render observations difficult to interpret.

Dr. S. B. Ekelman and Dr. Reynolds have reported the results of a study of the

effects of the analgesic drug Nisentil on patients in labor. The tokodynamometer, developed by Reynolds and his associates, was employed to evaluate the character of uterine contractions after administration of the drug. It is reported that Nisentil causes a decrease in the intensity of uterine activity in most primiparous women. It could not be stated whether the effects on the uterus are of sufficient duration and magnitude to delay the progress of labor.

#### HORMONES AND REPRODUCTION

##### *A Pharmacologic Block to Ovulation*

Dr. Vincent J. De Feo and Dr. Reynolds completed the initial phase of a program designed to evaluate the effects of the rauwolfia alkaloid reserpine on the reproductive cycle of the rhesus monkey. The approach was suggested by the observation by others that in rats ovulation may be suppressed when the drug is given in proestrus. It has been argued that reserpine interposes its functional block to ovulation through the hypothalamus. The results of these preliminary studies, which have been prepared for publication, may be summarized as follows: It was found that when the drug is administered for 100 to 110 days in a dose of 1 mg/kg (larger than is used clinically), menstruation is suppressed up to 140 days. Ovulation did not occur during treatment. The uterus was found to be hyperemic but in a resting state. The ovaries contained no corpora lutea, only numerous small follicles. The drug also influenced the cycle when given for a shorter duration, provided administration occurred at the beginning of a cycle, i.e. from the 2d to the 12th day inclusive. Under these conditions, ovulation did not occur (at least by the 23d day), and menstruation was suppressed from 60 to 90 days. No effect on ovulation was observed when the drug was given from the 8th to the 16th day of the cycle. These provocative observations require additional testing and controlled observation, and study of seasonal variations.

### *Deciduoma Formation in Relation to Hormonal Activity*

*Deciduoma Formation in Puberal Animals.* The ability of estrogens to suppress the development of a deciduoma produced by traumatization of the endometrium is being investigated in puberal rats by Dr. De Feo. Castration at the time of uterine traumatization, i.e. the 4th day of pseudopregnancy, must be followed by daily administration of progesterone in order to produce a normal deciduoma response. If, together with progesterone, estradiol is administered, a gradual inhibition is observed depending on the amount of estrogen employed. A ratio of 5000 parts of progesterone to 1 part of estradiol is sufficient to inhibit growth completely in most cases. Increasing the ratio of progesterone to estrogen produces better growth, but less than that achieved by progesterone alone. Dr. R. E. Nesbitt, of the Department of Obstetrics at the Johns Hopkins Hospital, who has histologically examined samples of the tissues obtained in these experiments, has reported that higher estrogen concentrations inhibit differentiation. As the estrogen level is reduced, the antimesometrial decidua appears, but the mesometrial decidua is not seen until the progesterone-to-estrogen ratio is 12,000 to 1 or higher. Deposition of intracellular glycogen in the mesometrial decidua parallels the growth of this region. Alkaline phosphatase, predominantly extracellular, increases in the antimesometrial decidua. Initial studies in adult pseudopregnant and pregnant rats reveal a similar pattern of macroscopic inhibition, in which a progesterone-to-estrogen ratio of 10,000 to 1 impairs deciduoma formation and pregnancy.

*Loss of Uterine Sensitivity.* Following the production of a massive deciduoma, in which a decidual reaction is elicited from the tubal to the cervical end of the uterus by traumatization by needle, a period of two to three weeks must elapse after the termination of the pseudopregnancy before the uterus is again capable of responding

to a trauma. This failure in deciduoma formation has been attributed to a loss in uterine sensitivity; the mechanism of the loss is under investigation.

Rats in which a massive deciduoma response has been obtained in one uterine horn are found to be sterile in that horn only. Part of the sterility may be due to a loss in uterine sensitivity to the blastocyst; most of the failure, however, may be attributed to a failure of fertilization resulting from a lack of continuity of the uterine lumen, which must be formed anew from the uterine glands after a massive deciduoma.

*Effect of Reserpine on the Reproductive Cycle of the Rat.* Since deciduoma formation serves as an index of progesterone activity and is, therefore, an index of the integrity of the pituitary-ovarian axis, it was of interest to test the effect of reserpine on this system. Administration of the drug during the first four days of pseudopregnancy depressed deciduoma formation. The impairment was not improved by the administration of progesterone during the pseudopregnancy. These findings suggest that a part of the inhibition of the deciduoma response must be attributed to an action of reserpine on the uterus through a mechanism that does not involve the ovary.

### *Ovarian and Gonadotropic Levels of Hormones in the Lactating Mouse*

There is considerable evidence in the literature that lactation produces a hormonal state which differs from that in pregnancy. In investigating the mechanisms concerned, Dr. Greenwald has conducted the following studies: Lactating mice have been sacrificed at one-day intervals during the month following parturition; the litters have been arbitrarily reduced to six young at birth. The uterus, ovaries, and vagina of each animal have been studied histologically for evidence of the presence of estrogen, progesterone, and gonadotropins. There is considerable in-



dividual variation among the lactating animals, but, in general, there appears to be a reduced release of gonadotropins from the pituitary during the first ten days after parturition. As a result of the low output of gonadotropins, there is no evidence of estrogen; progesterone, however, is present as determined by the Hooker-Forbes technique. Increased release of gonadotropins after ten days post partum is indicated by a spurt in follicular growth. Subsequently, estrogen is secreted in conjunction with progesterone, as evidenced by vaginal mucification. It is believed that the deficiency of estrogen as a result of decreased release of gonadotropins accounts for a number of phenomena, such

as delayed implantation and the prolonged period of uterine sensitivity to trauma during lactation.

#### *Ovarian Cycle of the Muskrat*

During the year, Dr. Greenwald has devoted a part of his time to a continuation of histological study of the muskrat ovary. The most exceptional feature observed is the conspicuous development of the epoöphoron, which is continuous with an equally well developed rete ovarii. The epoöphoron appears to undergo cyclic changes in size and secretory activity correlated with the female reproductive cycle, reaching its maximum development at the time of estrus.

### APPARATUS AND TECHNIQUES

Editors, editorial advisers to scientific journals, and others concerned with the ever increasing tide of scientific publications often have asked to what extent a given article is read by the followers of the journal in which it appears. Many of the articles published in the Contributions to Embryology are highly technical; often it is difficult even to review them without resorting to the specialized vocabulary of the author. Obviously they are of interest to a relatively small number of persons actively engaged in a related field of research. Volume 36 of the Contributions to Embryology, however, will contain one article which, it may confidently be expected, will be of interest to all its readers. The Staff of the Department of Embryology takes pleasure in presenting in this volume, as a tribute to Dr. Chester H. Heuser, an

illustrated description of the methods used by him in the preparation of early embryos. Dr. Heuser, Staff Member of the Department for twenty-nine years and Research Associate since his retirement in 1950, is renowned for his unusual technical ability and his unexcelled knowledge of the early mammalian embryo. It is fitting that Dr. Heuser's techniques have been described by Osborne O. Heard, Senior Modeler of the Department of Embryology, who, as a long-time associate and co-worker of Dr. Heuser, is well qualified to present the novel and precise procedures in such a manner that the reader is given an understanding not only of the details of the operations, but also of the special conditions or problems that led Dr. Heuser to adopt a particular course in dealing with his material.

### CO-OPERATIVE ACTIVITIES

During the year, Dr. Elizabeth M. Ramsey examined 116 specimens sent by 27 institutions and physicians from 14 cities in 9 states and Alaska. Reports were given to all the senders. Of the 116 specimens, 85 were discarded as being of no research or museum value, at the end of three months and in the absence of instructions

to the contrary. Two specimens were returned to the donors, and 29 specimens were in good enough condition to justify permanent preservation. There has been no decline, in recent years, in the number of specimens received at the laboratory. It is a pleasure to acknowledge that our colleagues have again contributed much



valuable, beautifully prepared material; one case in point is the interesting specimen contributed by Dr. A. Charles Posner, of Harlem Hospital, New York City, which was the basis of a paper by Dr. Ramsey entitled "Fetal Circulation in Anomalous Conditions (Fetus Amorphus Anideus)." In Dr. Ramsey's opinion, however, there is a clear trend toward lowered quality in the specimens examined. We stand ready to examine any specimen sent to the laboratory, but we confidently hope that, in the future, a preliminary screening by the donors will eliminate macerated and poorly fixed specimens that do not justify careful scrutiny from the standpoint of either pathological or research value.

The interested reader will have noted frequent reference throughout the text of this report to research done by members of the Department of Embryology in collaboration with investigators at the Johns Hopkins University and the Johns Hopkins Hospital. It is hoped that these cordial and mutually beneficial associations will be continued, and the ties between the Department of Embryology and the Department of Biology and the McCollum-Pratt Institute will be strengthened. The sig-

nificance of these relations has been recognized by the appointment of staff members of the Department to honorary positions in the University. At the time this report is written, two members of our staff hold appointments: for several years, Dr. Burns has held the title Professor of Biology; recently Dr. Ebert was appointed Professor of Embryology.

An example of a practical co-operative venture is the continued participation of the Department of Embryology in two investigations carried on by Dr. Lawrence R. Wharton, of the Johns Hopkins Hospital, Professor Richard W. TeLinde, of the Johns Hopkins Medical School, and Dr. Roger B. Scott, of Western Reserve University School of Medicine. The studies deal with the experimental production and treatment of endometriosis and with the effects of hysterectomy on the ovary in the macaque. The Department of Embryology participates in several ways—by furnishing facilities and care of animals and technical assistance, and by an exchange of data and ideas. Dr. Bartelmez and Dr. Ramsey, in particular, have been keenly interested in the research, and several combined operations have been carried out.

### STAFF ACTIVITIES

In order to widen their range of knowledge and interests, and to master new skills which should enable them to probe more deeply into their own specialties, several members of the staff traveled extensively. Dr. Burns was granted a John Simon Guggenheim Memorial Foundation Fellowship for travel to Europe in order to observe work in progress in the field of experimental control of sex differentiation in a number of laboratories, and, in particular, for a period of residence at the University of Paris, where important work in this field and in the endocrinology of development is pursued in several laboratories. He went to Paris in time to attend the Sixth Federative International Congress of Anatomy, July 25-30, 1955, where he presented a

paper entitled "Experimental Transformation of Testis to Ovary in the Opossum Embryo."

In Paris, Dr. Burns was appointed Exchange Professor in the Faculté des Sciences, with residence in the laboratory of Professor L. Gallien. In this capacity, he participated in a seminar course for advanced students organized by Professor Gallien, entitled "L'ontogénèse sexuelle des vertébrés." In addition, Dr. Burns visited the laboratories of Professors A. Jost, of the Faculté des Sciences, A. Raynaud, of the Institut Pasteur, and E. Wolff, of the Collège de France; in the latter laboratory, a study was made of the special methods developed for the culture of embryonic organs and organ primordia in vitro, particu-

larly the gonads of chick and mouse embryos. During a brief visit to England late in 1955, Dr. Burns gave lectures under the general title "Experimental Studies of Sex Differentiation in the Opossum" at the School of Anatomy, Cambridge University; Charing Cross Hospital Medical School, University of London; and the Department of Physiology, St. Mary's Medical School. Other appearances included a lecture given February 27, 1956, at the University of Geneva, entitled "L'action des hormones sur la différenciation sexuelle de l'Opossum," and a special lecture given first at the Société Zoologique de France and later before the Faculté des Sciences in the Sorbonne, on the subject "Vie et reproduction de l'Opossum américain."

With the aid of a grant from the National Science Foundation, Dr. Bishop undertook a trip to Europe during the spring of 1956. He presented papers at the Congress on Fertility and Sterility, held at Naples in May, and at the Congress on Animal Reproduction, in Cambridge in June, and gave lectures at several universities. After fulfilling these responsibilities, he began a stay of several months at the Max Planck Institute for Medical Research, in Heidelberg, where he has undertaken a collaborative investigation with Dr. Hoffman-Berling, under the general guidance of Professor H. H. Weber, in an attempt to isolate and characterize the nature of the contractile protein in sperm tails. On the basis of fragmentary, indirect evidence, it is frequently stated that the sperm tail contains an actomyosin-like protein. Identification of such material in these flagellated cells should open an interesting line of investigation.

It may be recalled that Dr. George W. Corner presented the annual Joseph L. Baer Lecture of the Chicago Gynecological Society on October 15, 1954. The published version of that lecture, entitled "The Observed Embryology of Human Single-Ovum Twins and Other Multiple Births," has now appeared. In this lecture, Dr. Corner reviews the embryological theory

for single-ovum twinning, and considers how far it is supported by observations of twin embryos and by relevant experiments on animals. The article is well illustrated with early cases from the Carnegie Collection of human embryos and from several other sources.

Dr. Vincent J. De Feo attended the annual meetings of the American Physiological Society, the Federation of American Societies for Experimental Biology, and the Endocrine Society. At the last-named meeting, he presented a paper entitled "Loss of Uterine Sensitivity in the Rat following the Production of Deciduoma."

Dr. Elizabeth M. Ramsey participated in the Third Microcirculatory Conference as a member of the Symposium Panel, and presented lectures before the Obstetrical and Gynecological House Staff at Columbia University College of Physicians and Surgeons and the Department of Zoology at Mount Holyoke College. On all these occasions, Dr. Ramsey dealt with aspects of the placental circulation. In addition, she gave a preliminary report of a study in fetal pathology at the annual meeting of the American Association of Anatomists.

Dr. Bent G. Böving and Dr. James D. Ebert also presented accounts of recent investigations at the meeting of the anatomists. In addition, Dr. Ebert lectured at Duke University on the subject "Some Aspects of Protein Biosynthesis in Development." He also participated in the Third Conference on Gestation held by the Josiah Macy, Jr. Foundation at Princeton, New Jersey. He continued during the year to serve as a member of the Advisory Panel on Genetic and Developmental Biology of the National Science Foundation, the Panel on Biology of Neoplasia of the Committee on Growth of the National Research Council, the Committee for Basic Biological Research on Aging of the American Institute of Biological Sciences, and the Executive Committee of the Society for the Study of Development and Growth. Dr. Ebert also participated in the

organization of the workshop in Developmental Biology held at Bar Harbor, Maine, June 18-28, 1956.

Dr. Reynolds presented lectures at the Brooklyn Academy of Pediatrics, the College of Medicine of the State University of New York, the University of Maryland

Medical School, and the Department of Obstetrics at the Walter Reed Army Medical Center.

Four prints by Richard D. Grill, photographer, were chosen for exhibition in the Biological Photographic Association Traveling Salon for 1955.

## CONTRIBUTIONS TO EMBRYOLOGY, VOLUME 36

At the time of this report, volume 36 of the Contributions to Embryology is in preparation. Publication is expected in 1957. The volume will contain the following articles:

242. Osborne O. Heard, *Methods Used by C. H. Heuser in Preparing and Sectioning Early Embryos*.

243. Benjamin C. Moffett, Jr., *The Prenatal Development of the Human Temporomandibular Joint*.

244. Chester H. Heuser and George W. Corner, *Developmental Horizons in Human Embryos. Description of Age Group X, 4 to 12 Somites*.

245. E. Ide Smith, *The Early Development of the Trachea and Esophagus in Relation to Atresia of the Esophagus and Tracheoesophageal Fistula*.

246. Perry W. Gilbert, *The Origin and Development of the Human Extrinsic Ocular Muscles*.

247. Dorcas H. Padget, *The Development of the Cranial Venous System in Man, from the Standpoint of Comparative Anatomy*.

248. E. Carl Sensenig, *The Development of the Occipital and Cervical Segments and Their Associated Structures in Human Embryos*.

249. George W. Bartelmez, *The Form and the Functions of the Uterine Blood Vessels in the Rhesus Monkey*.

250. Ronan O'Rahilly, D. J. Gray, and Ernest Gardner, *Chondrification in the Hands and Feet of Staged Human Embryos*.

251. Roy E. Crowder, *Development of the Adrenal Gland in Man, with Special Reference to Origin and Ultimate Location of Cell Types and Evidence in Favor of the "Cell Migration" Theory*.

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——— See CHINARD, F. P.

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## DEPARTMENT OF GENETICS

*Cold Spring Harbor, Long Island, New York*

M. DEMEREC, *Director*

Genetical research, like many other fields of endeavor, has its ebbs and flows. There are periods when, under the influence of some new idea, technique, or equipment, or through the use of a different experimental organism, a formidable barrier to the extension of our knowledge is swept away and new discoveries are made in rapid succession, until another barrier is reached and a period of meager returns and disappointments again sets in. It seems to me that research in genetics is now on the upswing, and, moreover, that this trend is being sustained by the breaking down of successive barriers and so is likely to continue for some time. New possibilities for the study of genetic mechanisms have been afforded by the introduction of microorganisms as experimental materials, and by a more extensive application of chemical and physical techniques to the solution of genetical problems. Similarly, innovations in laboratory methods for the study of population genetics have effectively contributed toward rapid progress in that branch of genetics.

This forward trend in genetics research has been reflected in the Cold Spring Harbor Symposia, which are organized by the Biological Laboratory and are an important function of our research center. The twentieth Symposium, held in the summer of 1955, presented exciting new developments in population genetics; and the twenty-first Symposium, in June 1956, revealed the wide approaches that are opening up for further analysis of the structure and function of genetic materials. It appears probable that methods now available will help to clarify the chemical aspect of the fine structure of genes, and that the morphology of genes and chromosomes will become more comprehensible through the application of electron-microscope tech-

niques. Members of the staffs of the Cold Spring Harbor laboratories are performing research in these areas of genetics, and took a prominent part in the last two Symposia.

### RESEARCH

Investigation of chromosomal elements that control gene action has been continued by McClintock, who is studying the types of such elements that function in the maize chromosome complement, and their specific modes of operation. A number of different controlling elements have now been identified. They fall into several categories with regard to mode of control of gene action, including inhibitors, mutators, and suppressors as well as other types of modifiers. Knowledge of the transposability of some of these elements has been considerably expanded. Probably the phenomenon of transposition is fairly extensive, for several different controlling elements are now known to undergo this process. Study of a structurally modified chromosome has also been undertaken, because in the presence of this modified chromosome new alterations in chromosome organization are produced. The types of alteration it induces are both unique and provocative. It also induces changes in gene action, and McClintock has begun an investigation of the mechanism responsible for these changes.

Kaufmann has shown by means of electron microscopy that the pattern of organization in dividing cells of *Tradescantia*, as in those of the salivary glands of *Drosophila*, is apparently represented by a hierarchy of pairs of helically disposed chromonemata. The somatic prophase chromosomes of the staminate-hair cells of these plants seem to be composed of at least 64 (and possibly 128) experimentally separable subsidiary units. Experiments

are now in progress to ascertain the nature of the materials or conditions that bind the bundle of strands into an aggregate which acts as a biological entity.

Kaufmann and his associates have also investigated the role of the chelating agent ethylenediaminetetraacetic acid (EDTA) in disrupting chromosomal integrity. The results of cytochemical analysis by Kaufmann and chemical analysis by McDonald, using *Drosophila* salivary glands and onion root tips, suggest that ribonucleic acid (RNA) is involved in this process either directly or indirectly. In addition, these investigators have demonstrated that immersion of growing onion roots in solutions of EDTA produces the same types of mitotic abnormality that are caused by immersion in ribonuclease or are induced by ionizing radiations. Moreover, Kaufmann and his collaborators have shown by genetic analysis that ribonuclease and EDTA can modify the frequency of crossing over in *Drosophila*. It has been concluded, therefore, that any agent that alters the normal metabolism of the cell, whether by changes in ionic environment or by removal of necessary cell constituents such as RNA, can modify chromosome form and behavior. The assumption of some workers that chelating agents exert their effects on the chromosomal fabric solely by the removal of calcium and magnesium ions, which serve as bridges between the macromolecular complexes of nucleic acid and protein of which the chromosomes are composed, is questionable.

Gay has extended the observations of nucleocytoplasmic relations in *Drosophila* by determining the stage in the development of the salivary-gland cell when blebbing of the nuclear membrane occurs. This process begins during the first half of the third instar period, and is correlated with the acquisition by the cell of a new secretory function, the production of secretion granules which later form a puparial glue. These findings give strong support to the hypothesis that the nuclear-membrane blebbing is a manifestation of a mecha-

nism for genetic control of cytoplasmic function. Technical procedures of enzymatic hydrolysis have been worked out for digestion of tissues fixed for electron microscopy. In analyses of the structures involved in the nucleocytoplasmic exchange, they have shown that deoxyribonucleic acid is included within the blebs and that ribonucleic acid constitutes part of the associated highly differentiated chromosomal material. The fine structure of salivary-gland chromosomes also has been studied by means of these techniques.

The results obtained by Hershey's group suggest that genetic replication and recombination in bacteriophage occur without extensive fragmentation of the parental chromosome. It has been demonstrated that after mixed infection with ultraviolet-irradiated and unirradiated phage particles large pieces of irradiated nucleic acid pass intact from parents to offspring. The recipient offspring particles are noninfective in consequence. A study has also been made of the way in which atoms of labeled phosphorus, introduced with one of the parents entering a genetic cross, reassort during genetic recombination. The reassortment is far from random, and suggests that part of the material transferred from parents to offspring is in the form of large, genetically specific pieces of nucleic acid.

Further analysis by Demerec's group of the structure and character of gene loci in *Salmonella typhimurium* indicates that mutants resulting from changes at different sites of a locus, although they are affected primarily in one function, may differ from one another with respect to various other properties, and that the sites of alleles having similar properties are not distributed randomly within a locus. The abortive-transduction method, as developed by Ozeki, has provided an extremely sensitive means of differentiating between adjacent loci governing similar phenotypes, and a powerful tool for the study of gene structure, since it enables us to obtain *trans* configurations in *Salmonella* with

considerable ease. Evidence from abortive-transduction experiments confirms the earlier identification of the *hiB*, *C*, and *D* loci based on transduction and biochemical tests, and indicates that all the mutants allocated to each of these three loci are allelic. On the other hand, these experiments have revealed that the twelve mutants previously assigned to the *hiA* locus in reality represent three closely linked loci (*hiA*, *E*, and *F*).

During the year, Moser completed a mathematical analysis of the dynamics of bacterial populations maintained in the chemostat. The theory he has developed, which is supported by experimental evidence, predicts the various patterns of population change in the chemostat, and the patterns of accumulation of infrequent mutant varieties in a population which at a certain time "changes over" from one strain to another, better "fitted" strain. It also presents a solution of the equations of growth for the chemostat, and discusses the steady states attained by homogeneous and heterogeneous populations. Furthermore, the theory provides the analytical background for calculation of bacterial mutation rates and specific-selection pressures, and of their standard errors, from data obtained in chemostat experiments.

#### STAFF

In April 1956 George Streisinger joined our group as a junior staff member. Streisinger's interest in genetics began when he was a high school student, assisting in Th. Dobzhansky's laboratory; and even before he entered college he carried on independent research in population genetics. As a graduate student under S. E. Luria he took up research with bacterial viruses, and continued that interest as a postdoctoral fellow at the California Institute of Technology. He has been studying the inheritance of ultraviolet sensitivity, and the genetic control of host-range and serological specificities, in phages T2 and T4 of *Escherichia coli*. He has found that

host-range mutants provide an excellent material for genetic analysis of the structure of the gene locus concerned, and for study of its mutability, and he is now working on these problems.

Mrs. Hilda H. Wheeler, who had been our librarian since 1943, retired at the end of June 1956. Mrs. Wheeler had been associated with the Department for many years, having first worked for Dr. C. B. Davenport in 1928. At the Eugenics Record Office, under Dr. H. H. Laughlin, her work included translations of scientific publications from the German, French, and Spanish, as well as critical, detailed analyses and computations of experimental data. Later, she acted as research assistant to Dr. M. Steggerda in his anthropological studies; and upon Dr. Steggerda's resignation from the Department she took charge of the library, where her extensive knowledge of scientific material and exceptional command of languages have been of special value to the Department. Mr. R. K. Thurston, superintendent of buildings and grounds for both the Department of Genetics and the Biological Laboratory since 1953, as well as Mr. David G. Reid and Mr. Oliver V. Rogers, Jr., of the maintenance staff, also retired during the summer of 1956.

During the past year the Department had the following fellows: Hermann Moser and Joseph D. Mandell, who held Carnegie Institution fellowships; Royston C. Clowes, Fellow of the Damon Runyon Memorial Fund; Derek A. Smith, King George VI Memorial Fellow; and Sibirgina Wagenaar, Fellow of the Research Scientists Program of the National Academy of Sciences-National Research Council. In the summer of 1956 Philip E. Hartman, before starting work at the University of Brussels as a Fellow of the American Cancer Society, spent two months at the Department continuing his analysis of histidine mutants of *Salmonella*, begun while he was here in 1954-1955. Harold Baer, of the Tulane Uni-



versity School of Medicine, collaborated with him in this work.

#### CO-OPERATIVE WORK

We have continued to work in close co-operation with colleagues engaged in genetical research at the Biological Laboratory of the Long Island Biological Association. During the past year B. Wallace and J. C. King continued their studies of population genetics with *Drosophila*, while E. Englesberg and P. D. Skaar worked in bacterial genetics.

We have also profited by association with visiting research workers who stayed at the Biological Laboratory for various periods of time. Among these were Mark Adams and Alan Bernheimer, of the New York University College of Medicine; K. Maramorosch and M. C. Niu, of the Rockefeller Institute for Medical Research; S. E. Luria and his associates from the University of Illinois; A. H. Doermann and his associates from the University of Rochester; A. Novick from the University of Chicago; H. Roman from the University of Washington; C. Yanofsky, of Western Reserve University; E. W. Caspari, of Wesleyan University; J. M. Buchanan from the Massachusetts Institute of Technology; R. Pritchard from the University of Glasgow; and H. Kacser and J. H. Sang from the University of Edinburgh.

Again this year Kaufmann and Gay received the co-operation of members of Brookhaven National Laboratory in connection with their use of the electron microscope at that Laboratory.

#### MEETINGS AND LECTURES

The twenty-first Cold Spring Harbor Symposium on Quantitative Biology, held in June 1956 at the Biological Laboratory, was attended by about three hundred scientists interested in studies of genetic mechanisms and in experimental embryology. About forty of the participants were from other countries.

In August 1956 a conference of scientists

engaged in research with bacterial viruses, organized by Hershey, was held at Cold Spring Harbor.

Weekly meetings of the research staffs of the Department of Genetics and the Biological Laboratory were held from October to May, for informal discussion of scientific problems of general interest and for reports of the current research of individual members. Seminar lectures were also scheduled each week throughout most of the year. They were attended by the scientific members of the Department and the Laboratory, and occasionally by scientists from near-by institutions. The speakers, who included staff members, members of the summer group, and invited guests, presented reviews of completed research problems in which they had made major contributions.

#### OTHER ACTIVITIES

During the year, 1023 cultures of *Drosophila melanogaster* were sent to high schools and colleges for use in the teaching of genetics. Mrs. G. C. Smith continued in charge of the *Drosophila* stocks.

The twenty-ninth issue of *Drosophila Information Service*, of which Demerec is editor, was prepared at the Department and distributed in January 1956. The Department continued to handle the preparation and distribution of the *Microbial Genetics Bulletin*, which is compiled and edited by Dr. Evelyn M. Witkin, of the College of Medicine of the State University of New York. Number 13 of the *Bulletin* was issued this year.

The library acquired 903 books, of which 80 were purchased, 26 were gifts or exchanges, 182 were bound periodicals, and 615 were from the former Genetics Record Office. Periodicals and serial publications regularly received numbered 356. Interlibrary loan service was requested from Brookhaven National Laboratory, Brooklyn Botanical Gardens, Cornell University, New York Academy of Medicine, Rockefeller Institute for Medical Research,



and the University of Missouri; whereas the U. S. Army, Bristol Laboratory, Rutgers University, and Chas. Pfizer Laboratories availed themselves of our inter-

library facilities. Approximately 463 books and periodicals were loaned during the year to staff members, assistants, and members of the Biological Laboratory.

## GROWTH AND INHERITANCE IN BACTERIOPHAGE

A. D. HERSHEY, ELIZABETH BURGI, JOSEPH D. MANDELL, AND NORMAN E. MELECHEN

Our major effort during the past year has again been aimed toward an understanding of nucleic acid (DNA) transfer from parental to offspring phage (see Year Book No. 53, 1953-1954, pp. 210-225). Recent work shows that all or much of the transfer is direct, in the sense that it does not involve breakdown below the polynucleotide level, and indeed that some of the intermediates are large, genetically specific fragments. Our experiments are concerned primarily with the functional significance of conservation during transfer. The independent studies of Levinthal and Stent and their collaborators measure the size of the conserved fragments of parental material among individual offspring phage particles. The several modes of attack complement one another in important respects, although a satisfactory interpretation of all the results is not yet possible.

Our work has again been partly supported by a grant (C-2158) from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service. Mandell is the recipient of a fellowship from the Carnegie Institution of Washington. Isotopes are supplied to us by the Oak Ridge National Laboratory, on allocation from the Atomic Energy Commission.

### EFFICIENCY OF TRANSFER

One of the striking facts about the transfer of DNA (labeled with  $P^{32}$ ,  $C^{14}$ , or  $N^{15}$ ) from parental to offspring phage is its low efficiency. Usually the offspring particles, when recovered as completely as possible, contain only about 40 per cent of the labeled atoms that their parents possessed.

By minor improvements in technique, we have recently increased the efficiency of transfer to about 60 per cent. More important, we believe that we can account for the losses in terms of the following facts.

First, some of the parental particles are noninfective because they fail to inject their DNA into the bacterium to which they attach. The proportion of such noninfective particles varies greatly from preparation to preparation. At best it amounts to 10 to 15 per cent of the particles.

Second, some of the particles inject improperly, with the result that their DNA ends up not in bacterial cells but in the form of acid-soluble materials in the culture medium. The nature of the accident causing this effect is unknown, but such DNA is clearly unavailable for transfer to offspring. The loss does not exceed 5 per cent in properly designed experiments.

Third, some of the infected cells lyse spontaneously about 25 minutes after infection. They release DNA in two forms, in offspring phage particles and in the free state. Neither form contributes to the over-all transfer measurement. They can be separately detected as follows.

The labeled phage particles released by premature lysis of some cells are promptly adsorbed to others. As a consequence, about half the labeled DNA they contain is broken down to acid-soluble material. This loss can be estimated from the sharp rise in acid-soluble  $P^{32}$  in the culture observed after 25 minutes. The remaining half of their DNA remains attached to bacterial debris after lysis, and can be measured from the parallel rise in  $P^{32}$  content of that fraction. The total amount of parental DNA lost in this way varies with

experimental conditions, but usually measures about 15 per cent.

The free DNA released into the culture medium by spontaneous lysis of some cells can be measured in various ways. The simplest way is to observe the difference in acid-soluble  $P^{32}$  in parallel cultures with and without added deoxyribonuclease. This measurement accounts for another 10 per cent of the parental DNA that fails to be incorporated into offspring.

The losses mentioned, together with the measured transfer, account for about 90 per cent of the parental DNA. We conclude that, if the transfer in a single productive bacterium infected with a single phage particle could be measured, a very efficient transfer would be observed. The losses encountered in actual measurements are due to trivial accidents that have nothing to do with basic mechanisms. Parental DNA shares common intracellular pools with newly synthesized DNA in infected bacteria. Both are efficient precursors of phage particles as long as they remain in the precursor pools.

This conclusion implies that the losses during transfer from parental to offspring phage are random losses. The same thing is suggested by the following facts. Maaløe and Watson showed that the transfer occurs with constant efficiency during successive cycles of growth, as it must if the losses are random. We found that the purine-pyrimidine composition of the transferred DNA is the same as that of the parental DNA (Year Book No. 53). Furthermore, the composition of the transferred DNA is independent of the presence of competing nucleosides added to the culture medium during transfer, suggesting direct transfer with random losses. We have repeated and extended the earlier experiments along these lines during the current year, with confirmatory results.

It seems clear that the bulk of the parental viral DNA is a potential precursor of offspring DNA. Probably little or none of the transfer occurs by way of intermediates smaller than polynucleotides. At least

part of the transfer conserves large, genetically specific structures, as the following summary shows.

#### TRANSFER OF RADIATION DAMAGE

Kozloff found several years ago that if bacteria are infected simultaneously with  $P^{32}$ -labeled, ultraviolet-inactivated phage particles and live unlabeled particles, so that each bacterium is infected with one or more particles of each kind, the resulting phage yield contains  $P^{32}$  derived from the irradiated particles. This finding seemed to divorce material transfer from genetic function.

We questioned this conclusion on two grounds. First, it was not clear whether the transfer under these conditions is normally efficient (Year Book No. 53), although we now believe that it is, as Kozloff supposed. Second, it was possible that offspring particles receiving radiation-damaged DNA from their parents might themselves be defective, in which case the genetic inferences would be quite different.

Only recently have we succeeded in obtaining sufficiently precise transfer measurements to decide these questions. The technical improvements, which are minor, need not be described here. The principles of measurement, and the results, are summarized below.

Several people had looked, by more or less direct methods, for noninfective offspring particles from bacteria infected with irradiated virus. Their attempts, like our own, always failed. The failure merely showed that the number of noninfective offspring per irradiated parent is small. It could not exclude the possibility that irradiated DNA is preserved intact from generation to generation in mixedly infected bacteria.

To test this possibility we asked whether the  $P^{32}$  derived from irradiated parental particles ends up in infective or in noninfective particles. The techniques that could answer this question were suggested by the properties of the irradiated parents themselves.

If bacteria are infected singly with  $P^{32}$ -labeled, ultraviolet-inactivated phage particles, no viral progeny are produced, and of course no  $P^{32}$  transfer is recorded. This is why the irradiated particles are said to be noninfective. If, however, bacteria are infected with the labeled, inactive particles plus live, unlabeled particles, the mixed progeny contains  $P^{32}$ . This shows that the inactive particles inject their DNA, which participates somehow in the mixed infection. The results of the two transfer measurements, in single versus mixed infection, provide a means of detecting  $P^{32}$ -labeled, inactive particles in a population containing active unlabeled particles, no matter how small the fraction of inactive particles.

This test was applied to the offspring from irradiated,  $P^{32}$ -labeled and live, unlabeled T2. It readily showed that much of the  $P^{32}$  transferred under these conditions was contained in noninfective particles.

The actual proportion depended on two main variables. The effect of exposure to radiation was maximal at about 10 times the average lethal dose as measured by plaque counts. Higher doses neither increased nor diminished the proportion of transferred  $P^{32}$  contained in noninfective particles. Varying multiplicity of infection was studied by using a fixed exposure to ultraviolet light, approximately 13 average inactivating doses. The effects observed were not large, but showed a trend of the following kind.

The result was not detectably dependent on number of live, unlabeled phage particles per bacterium, except that the bacteria had to be infected with at least one live particle if transfer was to occur at all. For mixed infections, the proportion of transferred  $P^{32}$  contained in inactive offspring particles varied from 40 per cent to 70 per cent as the number of inactivated parents varied from one to ten per bacterium. At the higher ratios, so-called multiplicity reactivation occurred; that is, one could get viable offspring even if the unirradiated parent was omitted. This had

no striking effect on the radiochemical result.

The first question to be asked was the following. Are the inactive offspring particles noninfective because they contain DNA derived from the irradiated parent, or because of spreading metabolic effects of the presence of irradiated DNA in the cells? This question was answered by introducing the label from the culture medium, or by infecting with irradiated, unlabeled parents and labeled, unirradiated parents. In either case the  $P^{32}$  in dead particles among the offspring amounted to only 0 to 30 per cent of the total  $P^{32}$  content as the number of irradiated parents per bacterium varied from one to ten.

The possible interpretations of this result can be illustrated by two alternatives. First, suppose that virus particles contain two kinds of DNA, one photosensitive and one not, as is suggested by the response to varying doses of ultraviolet light. All our results can be explained on the basis of this hypothesis if the photosensitive portion measures about 40 per cent of the transferred DNA and if the remainder is distributed at random between live and dead particles among the offspring. According to this interpretation, the label derived from the live parent and transferred to dead offspring measures the number of dead offspring, which in turn is dependent on the multiplicity of infection with the irradiated parent. The number of dead offspring is determined in addition by the efficiency of transfer and by the physical dispersion among the offspring of the irradiated parental DNA. The cause of death of the offspring is the incorporation into them of a piece of photosensitive parental DNA carrying one or more persistent lesions.

This interpretation is interesting because it suggests a functional differentiation of the viral DNA, presumably into a photosensitive chromosomal part and a photoresistant nonchromosomal part. It also suggests that the manner in which radiation effects are transmitted to offspring



particles could provide information about the manner in which parental chromosomes are transmitted.

The second interpretation is less interesting. It can be assumed that a dead offspring particle contains the intact DNA from one irradiated parental particle or none. Dead particles containing irradiated DNA represent parental material that has been entirely excluded from participation in growth. Those not containing irradiated DNA must be produced by an independent mechanism. The live particles containing irradiated DNA reflect complete erasure of the effects of irradiation. This interpretation fails to explain the response to radiation dosage. It has not been formally excluded, however.

#### ASSOCIATED TRANSFER OF PARENTAL ATOMS AND PARENTAL GENES

If one infects bacteria with a mixture of two genetically marked phages, for example the host-range mutant  $h$  and non-mutant  $h^+$ , the two types reappear among the offspring in the same relative numbers with which the bacteria were infected. In theory, the two types of phage among the offspring can be readily separated from each other, though there are practical difficulties that need not be discussed here. It is feasible, therefore, to measure the exchange of atoms between the two types of phage by using additional isotopic markers. The appropriate experiment may be thought of as an  $h \times P^{32}$  cross; bacteria are infected with  $P^{31} h$  and  $P^{32} h^+$  parents, and the  $P^{32}$  content of  $h$  and  $h^+$  offspring is measured.

Garen described a preliminary experiment of this type (Year Book No. 53, p. 214). He found that considerable amounts of  $P^{32}$  passed from particles of one genotype to those of another. We have studied this phenomenon more carefully. The results can be summarized as follows.

If bacteria are infected with equal numbers of the two parental phages (5 particles each per bacterium), approximately two-

thirds of the conserved isotope is found in offspring particles having the genotype of the labeled parent. The result is independent of the genotype of the radioactive parent.

This result can be interpreted in at least three ways. First, one can suppose that the parental genetic material is fragmented by genetic recombination, in which case only a certain fraction will remain in association with a single genetic marker. Second, one can suppose that the parental nucleic acid is of two kinds, one kind inseparably associated with a single genetic marker, the second transmitted independently of the genetic marker. Third, one can suppose that the transfer involves two mechanisms, one that conserves the original association between atoms and genotype, and one that does not. These three hypotheses make different predictions concerning the experiments described below.

In the following experiment, we test the effect of altering the relative input of the parental phages. Bacteria are infected with an average of 0.5 particle of  $P^{32} h$  phage and 10 particles of  $P^{31} h^+$  phage. This has the effect of greatly increasing the fragmentation of the genome of the  $h$  parent as tested by conventional genetic experiments. Nevertheless, we find about one-third of the conserved  $P^{32}$  atoms associated with the  $h$  particles among the offspring. This result tends to exclude genetic recombination as the sole cause of redistribution of label.

In another experiment, we test the effect of repeated cycles of mixed growth. Bacteria are infected with 5 particles each of  $P^{32} h$  and  $P^{31} h^+$  phage. Two-thirds of the  $P^{32}$  in the offspring is found in particles of the  $h$  genotype. The mixed offspring particles are then used as parents during a second cycle of growth, following infection with 10 particles per bacterium. Of the  $P^{32}$  conserved among the second-cycle offspring, only 59 per cent is contained in particles of the  $h$  genotype. This result tends to exclude the second hypothesis stated above, unless it is assumed fur-



ther that the two hypothetical classes of nucleic acid are conserved with different efficiencies.

A third experiment, employing two or more genetic markers and permitting analysis of recombinant genotypes for  $P^{32}$ , is feasible in theory but we have not yet succeeded in carrying it out.

It should be admitted that the experimental errors in these measurements may preclude any detailed inferences. Only one fact is clear: all the results show that an appreciable amount of parental DNA remains associated with the parental genotype during transfer.

#### DISCUSSION

Our experiments show clearly that DNA can pass in functionally intact form from parental to offspring phage. The experiments with irradiated parents suggest further that only a part of the parental DNA is conserved in this form, and that the specifically conserved pieces are large in size and few in number relative to the number and DNA content of irradiated parental particles.

The experiments of Stent and his collaborators, employing entirely different

methods, also suggest conservation of parental DNA partly in the form of large pieces, and further suggest that these pieces are not subject to progressive fragmentation during viral growth. The function of these pieces is unspecified except that phage particles containing them are subject to inactivation by decay of incorporated radiophosphorus.

Levinthal has introduced a very powerful method of studying these questions. In effect, he can measure the size of a single piece of DNA down to an equivalent molecular weight of about 10 million. His experiments show that phage particles contain large pieces of DNA, and that these are conserved more or less intact among the offspring. These facts raise all the questions discussed in this report. Levinthal's method, radioautography of  $P^{32}$ -labeled DNA, supplements other physical methods in an indispensable manner.

It is to be hoped that results obtained by the various methods mentioned above can be fitted into a single scheme. Such a scheme would necessarily say something about possible mechanisms of genetic replication and recombination. For this reason the need for further experiments along current lines is acute.

#### BACTERIAL GENETICS

M. DEMEREC, H. MOSER, R. C. CLOWES, E. L. LAHR, H. OZEKI, AND W. VIELMETTER

The efforts of our group have been focused on continued analyses of gene structure, using the bacterium *Salmonella typhimurium* as material. We have been able to determine that mutations at different sites of a gene locus, although they affect one function primarily, may produce mutants that differ considerably from one another with respect to various other properties, and, moreover, that the sites of alleles having similar properties are not distributed at random within a locus but tend to be grouped together. The development of a method utilizing the phenomenon of "abortive transduction" has enabled us to obtain *trans* configurations of closely

linked similar mutants, and to study their allelic relationships. Results of our research during the past two years have been described in nine papers published collectively, under the title *Genetic Studies with Bacteria*, as Carnegie Institution of Washington Publication 612. Moser has completed a mathematical analysis of mutability in certain bacterial populations, which also has been submitted for publication in the Institution series.

In addition to the workers named above, our group included Dr. Sheila Howarth, of the Chester Beatty Research Institute, Royal Cancer Hospital, London, and Mr. Božidar Djordjević, of the Institute of

Nuclear Sciences "Boris Kidrich," Belgrade, both of whom joined us in May 1956. Mrs. Jean W. McIntyre continued in charge of washing and sterilizing of glassware, and Mrs. Emmy M. Snyder in charge of the preparation of culture media.

During the summer of 1956, Dr. Philip E. Hartman, before taking up an American Cancer Society Fellowship at the University of Brussels, continued his studies of histidine mutants here, in collaboration with Dr. Harold Baer, of the Department of Microbiology, School of Medicine, Tulane University. Dr. Charles Yanofsky, of the Department of Microbiology, School of Medicine, Western Reserve University, and Mr. Takashi Yura, of the Department of Microbiology, Yale University, took part in the analysis of tryptophan mutants.

Dr. Royston C. Clowes, of the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, was here throughout the year as a Fellow of the Damon Runyon Memorial Fund; and Mr. Derek A. Smith, of Reading University, England, spent the academic year 1955-1956 with us as a King George VI Memorial Fellow.

#### ABORTIVE TRANSDUCTION

A process known as "abortive transduction," responsible for the "trail phenomenon" in certain instances of transduction of motility in *Salmonella*, was described by Stocker, Zinder, and Lederberg. A comparable case has been studied by Ozeki in adenine-thiamine (*ath*) auxotrophs of *S. typhimurium*. In this case phage-transported genetic material fails to be incorporated into the genome of the recipient cell, and cannot duplicate, but persists in one of the progeny, resulting in the formation of a "minute" colony. This interpretation was based on the following observations.

In transduction experiments involving certain *ath* bacteria and phage carrying the wild-type allele of these mutants, very small colonies appeared in addition to the

expected large colonies when the infected bacteria were plated on adenine-pantothenate medium. When these minute colonies were picked up from the plates with a wire loop and streaked on fresh plates, a single minute colony appeared on each streak. In no case did more than one minute colony appear on any one streak. Furthermore, the results of the streaking experiment were confirmed by means of Newcombe's respreading technique; the number of minute colonies did not increase after respreading. These findings suggest that at any one time each minute colony contains only a single cell capable of producing another minute colony, and that this cell probably carries as an extra factor the phage-imported wild-type allele of the recipient marker. Transduction experiments indicated that almost all the constituent cells of a minute colony are genetically identical with the original recipient bacterium.

In further experiments, phage-infected bacteria were plated on minimal medium and incubated for various lengths of time before addition of adenine and pantothenate to the plates. Minute colonies were not detectable during the preliminary incubation, but they appeared and grew rapidly after addition of the supplements. Their final size was not affected by the length of the preliminary incubation period on minimal medium. It may be inferred from these observations that a cell carrying an extra wild-type allele is prototrophic in phenotype and consequently can divide on minimal medium; that the one daughter cell which does not receive the extra factor has a modified phenotype that enables it to undergo a finite number of divisions on adenine-pantothenate medium; that this phenotypic modification persists through 16 to 18 divisions; and that finally all the progeny resume the typical phenotype of the recipient cell (adenine-thiamine requirement).

Minute colonies appeared only in experiments in which the donor was either wild type or a mutant belonging to a

group different from that of the recipient, not in experiments involving mutants of the same group. Since members of a group are nonidentical alleles of the same locus, these results suggest that transfer of the complete wild-type locus is necessary for the appearance of minute colonies. Thus it seems that the phenomenon of abortive transduction reflects a complementary functional relation between the marker genes of the recipient and the donor. If it is a general phenomenon, the technique developed by Ozeki could be applied in tests of allelism. In a bacterium carrying an unincorporated chromosome fragment, the two mutant genes are in *trans* configuration, and therefore the presence or absence of minute colonies in transduction experiments involving two phenotypically similar mutants would provide information regarding the functional unity of these mutants. Experiments now under way with tryptophan, histidine, cystine, and other auxotrophs indicate that minute colonies can be detected whenever the rate of residual growth of the recipient bacteria is not too high to obscure them. Thus it seems probable that the phenomenon of abortive transduction is a general one.

The frequency of occurrence of abortive transduction has always been found to be higher, roughly by a factor of 10, than that of stable transduction; and an abortive transduction usually does not change into a stable one. Recently it was found that ultraviolet irradiation of the donor phage reduces the frequency of occurrence of abortive transduction, although, as previously reported by Garen and Zinder, the frequency of occurrence of stable transduction is increased by using ultraviolet-irradiated phage. This finding might be explained in the following way. Abortive transduction may require a longer portion of the transducing fragment, because the presence of a complete functional wild-type locus in the unincorporated fragment is necessary for minute-colony formation, whereas stable transduction requires only the small portion of the locus correspond-

ing to the mutated site. Moreover, in abortive transduction the unincorporated fragment is necessarily maintained during successive generations, with no possibility of "rescue" by recombination. In addition, treatment with ultraviolet may facilitate integration of the transducing fragment into the genome of the recipient cell, thus effecting an increase in stable transduction and a concomitant decrease in abortive transduction.

#### ANALYSES OF AUXOTROPHS

*Methionine mutants.* Genetic analysis of a group of nutritionally deficient mutants is greatly facilitated if the pathway of biosynthesis of the end product involved in the deficiency is known, and if intermediate compounds are available for testing. Under such conditions, through the application of both genetic and biochemical criteria, the gene loci represented by a group of auxotrophs can be identified with a fairly high degree of precision. On the other hand, lack of this biochemical information is a considerable handicap. Since the biochemical steps in the synthesis of methionine by *Salmonella* are not clear, our analysis of methionine-requiring mutants is being pursued under such a handicap, and any conclusions drawn as the work proceeds should be considered tentative.

During the past year Smith continued this analysis of methionine mutants, now 48 in number. He confirmed the previous finding by Glover (Year Book No. 54, pp. 223-224) that at least five gene loci are represented, namely, *meA*, *B*, *C*, *E*, and *F*. First, by testing possible intermediates in the biosynthetic pathway, Smith found that *meA*, *B*, and *F* mutants grow only on methionine, *meC* mutants are able to utilize either methionine or homocysteine thiolactone, and *meE* mutants can grow on either methionine or vitamin B<sub>12</sub>. Discrepancies between his and previous findings were traced to impurities in the chemicals used earlier. Feeding tests then



showed that *meC* mutants feed *meA* and *B* but not *meE* and *F*. No other feeding was demonstrable. Thus on the basis of utilization and feeding tests these mutants fall into four groups (*A-B*, *C*, *E*, and *F*); and it appears probable that the position of the *meC* block in the biosynthetic pathway follows those of the *meA* and *B* blocks and probably precedes those of *meE* and *F*. Further transduction tests confirmed the grouping and also distinguished between the *meA* and *meB* mutants. No evidence of linkage was observed between any of the five *me* loci. Thus, 48 methionine mutants represent five gene loci as follows:

*me A*-6, -7, -10, -15, -18, -19, -21, -22, -40, -43, -44, -48, -53, -54, -55;  
*meB*-16, -17, -20, 23, -33, -34, -36, -39, -41, -45, -49, -63, -66;  
*meC*-30, -32, -35, -37, -50, -52, -56, -57, -58, -65;  
*meE*-2, -47, -51, -60, -61, -62, -68;  
*meF*-31, -59, -64.

**Cystine mutants.** The analysis of cystine-requiring mutants was continued by Clowes. It was reported last year that the nutritional requirements in certain mutants could be satisfied by either sulfite or thiosulfate, and that on the basis of nutritional tests and transduction experiments 44 mutants had been divided into four groups: *cysA*, *B*, *C*, and *D*. More extensive transduction tests have revealed a fifth group, which is designated *cysE*. The nutritional requirement can be satisfied in *cysA* mutants by sulfite, in *cysD* by thiosulfate, and in *cysC* by either of these two compounds. In *cysB* and *cysE* mutants, no other inorganic or organic sulfur source investigated, except lanthionine, can replace cysteine at the usual concentration (10 µg S/ml). However, when the concentration of thiosulfate was raised very much higher (1 mg S/ml) than the normal equivalent of cysteine usually adequate for optimum growth, both *cysA* and *cysB* mutants were able to utilize this compound as a sole sulfur source. Concentrations lower than 1 mg S/ml did not

elicit growth, nor did increase of sulfite concentrations permit growth of mutants that do not otherwise utilize sulfite (*cysD*, *cysB*, *cysE*).

Syntrophism tests by the usual method of streaking pairs of mutants in parallel on solid enriched minimal agar showed positive results only after 5 days' incubation at 37° C, and these were restricted to the ability of certain *cysD* mutants to feed all *cysC* and *cysA* mutants. Auxanographic tests of disrupted concentrated cell suspensions of mutants confirmed this finding, and in addition showed that *cysA* mutants can feed *cysC* mutants. Since the corresponding supernatants were inactive and the cells were disrupted in approximately 500 times their volume, it seems likely that the growth-stimulating compounds which are accumulated in the cytoplasm cannot penetrate the cell wall. This inability might well explain the delay in syntrophism observed on solid medium as being due to the need for sufficient aging of the cultures to bring about autolysis and liberation of the accumulated product.

The biochemical data are consistent with the pathway diagrammed in figure 1, which would allow reduction of sulfite ( $\text{SO}_3^{--}$ ) by alternative routes in *S. typhimurium*. One pathway is by reduction to the hypothetical sulfoxylate ( $\text{SO}_2^{--}$ ), which may undergo spontaneous dimerization to thiosulfate ( $\text{S}_2\text{O}_3^{--}$ ) or may react with more sulfite to form hydrosulfite ( $\text{S}_2\text{O}_4^{--}$ ), which is converted by further sulfoxylate to a more reduced form of sulfur (shown as *X*). The reactions known to proceed in the absence of enzyme are shown by interrupted lines, and correspond to those reactions which are not apparently blocked in any mutants so far isolated. Growth curves of *cysD* mutants grown with hydrosulfite as a sole sulfur source and those of *cysA* mutants grown with either sulfite or hydrosulfite as a sole sulfur source suggest that the pathway utilized is different from that used in the wild-type strain or in *cysC* mutants growing on



either sulfur source. The inorganic ions may be held inside the cell by some large organic radicals, as postulated by R. B. Roberts and his colleagues in their studies with *Escherichia coli*. Since cysteic acid does not appear to be able to support the growth of even wild-type *S. typhimurium*, and cysteine sulfinic acid elicits the same growth responses as sulfite in all mutant and wild-type *S. typhimurium*, it seems likely that the *R* groups shown in figure 1 do not correspond to the cysteine carbon chain. Moreover, the inorganic compounds are effective competitors of sulfate utiliza-

cation that other groups are not closely linked. More exhaustive investigations of linkage were made, utilizing the biochemical differences between groups. When phage from a mutant of one group is used to transduce a mutant of another group, if there is linkage between the two groups some transductions should occur in which the fragment carried by the phage includes both the wild-type site corresponding to the recipient marker and also the donor mutant site, giving rise to cells with the donor genotype in addition to wild-type cells. If the donor type can utilize some

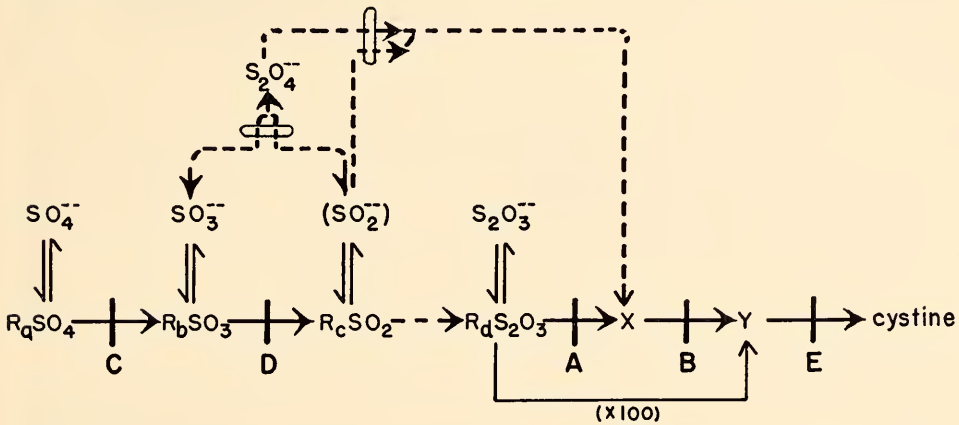


FIG. 1. Hypothetical pathway of cystine biosynthesis in *Salmonella typhimurium*

tion, as is shown by isotopic competition experiments, some of which were carried out with the co-operation and assistance of Drs. R. J. Britten and E. T. Bolton, of the Department of Terrestrial Magnetism.

The blocks shown at A, B, C, D, and E in figure 1 represent five groups of mutants whose nutritional character is consistent with a block at one of these positions. The mutants in each biochemical group correspond to those of one transduction group, except that mutants blocked at C and D form one closely linked transduction group. Apart from *cysC* and *cysD* mutants, the frequency of transduction between mutants of different groups is of the same order as that found between the same mutants and the wild type, an indi-

intermediate that does not allow growth of the recipient type, this compound when added to the selecting medium on which transduction platings are made will permit isolation of these cells. They are then identified by their inability to be replicated on a minimal medium. All the possible combinations among groups indicated lack of linkage between any except *cysC* and *cysD*, in which case the donor-type cells were almost ten times as frequent as wild-type cells. Linkage with the *try* group of markers, which has previously been demonstrated with certain *cys* mutants, was also investigated by this method and by the method of transduction of doubly auxotrophic cells (*try cys*) with phage from wild-type cells. All *cysB* mu-

tants showed linkage with one or another *try* marker, but no mutants of other *cys* groups showed such linkage.

It seems, therefore, that cystine biosynthesis is controlled by at least five gene loci arranged in four unlinked groups. One locus (*cysB*) is linked to the *try* series of loci, and two others (*cysA* and *cysE*) have so far not demonstrated linkage either with each other or with any other locus. The remaining two loci (*cysC* and *cysD*) show close linkage to each other. It is of interest that these two loci are concerned in sequential biochemical reactions, according to the scheme suggested in figure 1.

binants in tests with *hi-42*, *-45*, or *-44*. The detectable occurrence of abortive transductions in tests between *hi-41* and mutants of other loci indicates that its mutational site does not extend outside locus *F*. All these mutants produce tiny colonies of various sizes on minimal medium in the absence of transduction; these microcolonies can be distinguished from the "minute" clones produced by abortive transduction.

Group *B* is composed of strains whose mutational sites appear to be arranged in the order *hi-29*, *-20*, (*-40*, *-47*), (*-12*, *-24*), *-14*, *-21*. The fact that mutant *hi-22* produces no prototrophic recombinants in tests with any of these strains, but does partici-

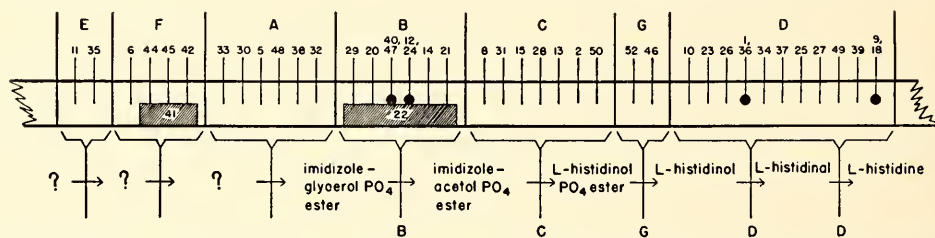


FIG. 2. Linkage map and proposed biochemical sequences for L-histidine synthesis in *Salmonella typhimurium*.

**Histidine mutants.** As reported last year (see fig. 1, Year Book No. 54, p. 222), biochemical and genetic tests placed 34 histidine-requiring mutants in four groups, *A* to *D*. Hartman, with the collaboration of Dr. Harold Baer, has extended the observations of these mutants and of several newly isolated strains by carrying out genetic tests for allelism by the abortive-transduction method.

On this basis, the mutants formerly placed in group *A* as nonaccumulators of imidazoles have now been divided into three groups: the closely linked strains *hi-11* and *-35* are members of a new group, *E*; *hi-6* and the new mutants *hi-41*, *-42*, *-44*, and *-45* are nonidentical alleles of a second new locus, *F*; mutants *hi-33*, *-30*, *-38*, *-48*, *-5*, and possibly *-32* now comprise group *A*. The most probable linear order of the mutant sites is shown in figure 2. Mutant *hi-41* gives rise to no prototrophic recom-

pate in detectable abortive transduction with members of the adjacent loci *A* and *C*, indicates that the region "covered" by the *hi-22* mutation does not extend outside locus *B*. According to recombination tests, *hi-40* and *-47* are extremely closely linked, if not identical, alleles. Mutants of group *B* are presumably deficient in the capacity to form sufficient imidazoleglycerol phosphate ester dehydrase. Activity of this enzyme has been detected in extracts of LT-2 wild-type *Salmonella* cells (Bruce N. Ames, personal communication).

Group *C* mutants, *hi-8*, *-31*, *-15*, *-28*, *-13*, *-2*, and *-50*, give no evidence of abortive transduction in any of the possible intra-group combinations but do produce abortive transductions in tests with mutants located outside group *C*. These, and all other abortive transductions among histidine mutants, give rise to acentric, micro-

scopic colonies of rather uniform size on minimal medium. In addition, when transduction plates bearing the temperature-sensitive *hi-15* recipient are moved to room temperature, the minute colonies slowly become visible to the naked eye. Restreaking experiments have verified Ozeki's general conclusions about the pattern of inheritance in abortive transduction. Group *C* mutants presumably lack a transaminase, found by Ames in extracts of wild-type cells.

Two of the new mutants, *hi-46* and *-52*, are closely linked to each other and give rise to abortive transductions with members of all the other groups. These two strains are both slightly "leaky," producing tiny colonies of various sizes on minimal medium. They respond to L-histidinol with a growth rate much more rapid than that of any of the other mutants that can utilize this intermediate. They are closely linked with some group *C* and group *D* mutants, and appear to represent a new locus situated between *C* and *D*. They have been designated group *G* mutants. The presumed function of locus *G* is the production of L-histidinol phosphate ester phosphatase, an enzyme found by Ames in extracts of wild-type cells.

Members of group *D* were characterized in last year's report; although tests have been limited, abortive-transduction analysis so far confirms their placement in a single locus, whose function is production of the enzyme histidinol dehydrogenase. Two new mutants, *hi-39* and *-49*, fulfill biochemical criteria for placement in group *D*; they are closely linked with *hi-9* and *-18*.

It is concluded that the histidine region of the *Salmonella* chromosome is divided into seven functional genes, or loci. Biochemical criteria cannot yet differentiate among several of the loci (*E*, *F*, and *A*) distinguished by abortive-transduction tests. All data are consistent with the conclusion that the loci are arranged in precisely the order determined for the pre-

sumed metabolic blocks in the primary biosynthetic sequence.

The high efficiency of stable transduction to prototrophy that is characteristic of all histidine mutants is paralleled by a similarly high frequency of abortive transduction. This finding, along with data concerning other mutants, indicates that the same genetic element that is involved in stable transduction (or a "gene product" attached to this element) is involved in abortive transduction. Preliminary data, obtained by Hartman in collaboration with Ozeki, indicate that the inactivation of abortive transduction of *hi-22* by ultraviolet irradiation of the transducing phage exhibits a one-hit curve, whose slope is identical with that of the inactivation of plaque formation by the phage. Stable transduction of *hi-22* is much less sensitive to ultraviolet inactivation. Thus the functioning of the *B* locus carried by the abortively transducing fragment seems to be more sensitive to ultraviolet than its "rescue" by recombination with the host genome.

*Aromatic amino acid mutants.* On the basis of their nutritional requirements, mutants defective in the synthesis of aromatic amino acids can be divided into four groups, as follows: (*a*) those whose requirement can be satisfied by tryptophan (*try*), (*b*) those that can grow on tyrosine (*tyr*), (*c*) those that can grow on phenylalanine (*phe*), and (*d*) those that require several compounds for growth (*arom*). Tryptophan mutants have been extensively investigated, and the results have been discussed in previous reports. During the past year Vielmetter began an analysis of ten mutants belonging to the other three groups.

Four tyrosine mutants (*tyr-1*, *-3*, *-4*, and *-5*) are able to grow on tyrosine but not on phenylpyruvic acid, which is one of the precursor compounds in the biosynthesis of aromatic amino acids. Transduction tests revealed that these four mutants are linked together; that is, they either are



nonidentical alleles of one locus or represent closely linked loci.

The deficiency exhibited by the single phenylalanine mutant (*phe-3*) in our collection can be satisfied either by phenylalanine or by phenylpyruvic acid. No linkage between *phe-3* and the other mutants was observed.

Five mutants (*arom-1*, -2, -3, -4, and -5) require several compounds in order to grow. The *arom-1* mutant requires tryptophan, tyrosine, phenylalanine, and *p*-aminobenzoic acid. Either anthranilic acid or indole is able to substitute for tryptophan, and phenylpyruvic acid can take the place of phenylalanine. Shikimic acid cannot support growth of this mutant. The remaining four *arom* mutants require both tyrosine and phenylalanine for growth. Transduction tests showed that *arom-3* and -4 are closely linked, whereas no linkage was detected between either *arom-2* or *arom-5* and any of the other *arom* mutants. Thus at least four gene loci are represented by these five mutants, which are designated as follows: *aromA-1*, *aromB-3* and -4, *aromC-2*, and *aromD-5*.

Feeding experiments showed that growth of *aromA-1* cannot be supported by accumulation products of any of the tryptophan mutants. Mutants *phe-3*, *aromB-4*, and *aromD-5* feed *tyr-1*, -3, -4, and -5; and *phe-3* feeds *aromB-3* and -4 and *aromD-5*.

In reviewing the biosynthesis of amino acids, Dr. B. D. Davis (*Advances in Enzymology* 16:247-312, 1955) proposed a scheme for the pathway of biosynthesis of aromatic amino acids (p. 288). Within this scheme, the block represented by *aromA-1* can be tentatively placed either before or after shikimic acid. In favor of the position before shikimic acid is the fact that spectrophotometric examination of medium in which the bacteria were grown revealed a peak with low extinction at 3100 Å, an indication that *aromA-1* accumulates dehydroshikimic acid. In favor of the position after shikimic acid is the observation that this compound is not able to support growth of *aromA-1*. A

finding by Davis in work with *Escherichia coli*, however—that the utilization of shikimic acid is inhibited in the presence of dehydroshikimic acid—supports the assumption that the *aromA-1* block is before shikimic acid. The probable locations of the three blocks associated with loci *aromB*, *C*, and *D* are before prephenic acid, on the branch of the pathway leading toward phenylalanine and tyrosine. The two remaining blocks are probably located beyond the point at which this branch splits into two parts: the *phe-3* block on the phenylalanine part, before phenylpyruvic acid, and the *tyr* block on the part leading to tyrosine synthesis, presumably before hydroxyphenylpyruvic acid.

*Tryptophan mutants.* Cell-free extracts prepared by sonic vibration from the six available *tryD* mutants as well as from the wild-type strain LT-2 were assayed for tryptophan synthetase activity by Yura. This activity was measured in terms of indole disappearance by the method developed by Yanofsky with *E. coli*. The wild-type extract showed high activity of the enzyme, whereas none of the *tryD* mutant extracts showed any detectable activity. The findings establish that these six members of the *tryD* group are deficient in a single enzyme activity. The minimum level of enzyme that could have been detected in this assay is about 5 per cent of that of the wild type.

Yura has made a study of abortive transduction in the six *tryD* mutants. The size of minute colonies due to abortive transduction was markedly increased when the minimal agar was supplemented with 0.02 to 0.05 per cent neutralized vitamin-free casein hydrolyzate. Phage grown on the wild type, or on strains carrying the markers *tryA-8*, *tryB-4*, and *tryC-3*, all closely linked to the *tryD* locus, caused minute-colony formation with about the same frequencies. All possible donor-recipient combinations (including reciprocal combinations) within the *tryD* group were tested for production of minute colonies. The results of several experiments clearly showed



that minute colonies were not formed in any of these cases. The available data therefore suggest that the complete wild-type allele of the *tryD* locus must be present in phage-carried genetic material for the formation of minute colonies, which is presumably brought about through restoration of an ability to synthesize a single enzyme, tryptophan synthetase.

A similar study using the abortive-transduction method has been made by Lahr with six mutants originally considered to be alleles of the *tryB* locus. On the basis of his results it appears probable that about three closely linked loci, all governing related steps in the biosynthesis of tryptophan, may be present in that region of the chromosome. This conclusion is receiving support from studies of enzyme activity being carried out with the same material by Dr. C. Yanofsky.

*Purine mutants.* Ozeki has continued the study of purine-requiring mutants carried on during the previous year by Yura (Year Book No. 54, pp. 227-229). Forty-three mutants have been allocated to four adenine-thiamine groups (*athA*, *B*, *C*, and *D*) and four adenine groups (*adA*, *B*, *C*, and *E*), distinguishable from one another either genetically or biochemically by criteria described in last year's report. In addition, another purine mutant (*guA-1*) has been isolated, which responds only to guanine or diaminopurine. The group formerly designated *adC* has been divided in two on the basis of data from transduction studies, although its members have similar biochemical properties as indicated by the compounds they accumulate and the nutrients they require for growth. Thus two gene loci have been identified in this biochemically similar group. One, which will continue to be called *adC*, is represented by two non-identical alleles (*adC-2* and *-7*), and the other, now designated *adE*, by the remaining nineteen nonidentical alleles (*adE-4*, *-5*, *-6*, *-8*, *-9*, *-11*, and *-14* through *-26*). The *adC* locus is linked with the *athC* and *guA* loci, whereas *adE* is not.

Further study has modified a previous conclusion that mutant *ad-10* is the single representative of a locus (called *adD*) which is closely linked with the *athD* locus. In extensive transduction experiments, using about  $10^9$  cells of *athD-12* bacteria and  $10^{10}$  phage particles grown on *ad-10* cells, Ozeki found about 1000 donor-type colonies but no prototrophs. This result indicates that recombination does not occur between *athD-12* and *ad-10*, and can be explained by assuming either that these two mutants are due to changes at homologous sites of a gene locus, or that one or both of them involve changes at several adjacent sites, at least one of which is homologous for both mutants. Since a considerable diversity of phenotypes is observed among purine mutants, the phenotypic difference between *athD-12*, which requires adenine and thiamine, and *ad-10*, which can grow on adenine alone, is very likely due to this diversity. Experiments were made to test another possibility, namely, that *ad-10* is an identical allele of *athD-12* but carries a suppressor which modifies its phenotype. The results were negative. An analysis of culture fluids of *ad-10* and members of the *athD* group showed similar absorption curves in the ultraviolet region of the spectrum, which is further evidence in support of the assumption that *ad-10* is a member of the *athD* group rather than a representative of another gene locus. Thus, in our future work this "*ad-10*" mutant will be considered an allele of the *athD* locus, and will be designated by the new symbol *athD-20*.

Further studies have been made concerning the linkage relations among the nine purine loci. Linkage is indicated when in transduction experiments between members of two loci the donor phenotype can be detected. In each such case, the genotype of cells exhibiting the donor phenotype was confirmed by further transduction tests. The results revealed two linkage groups, namely, *adC-athC-guA*, in the order listed, and *athD-athB*. The numbers of prototrophs obtained in

experiments with loci of the first group were considerably larger than the numbers obtained in *athD-athB* combinations.

#### VARIATION AMONG SITES OF THE SAME LOCUS

It has been observed that nonidentical alleles of the same gene locus often differ as to frequencies of mutation to prototrophy, either spontaneous or induced by treatment with ultraviolet radiation or other mutagens. Differences have also been observed in the reactions of alleles to various nutritional factors. During the past year Clowes and Ozeki have made studies of biochemical differences among mutants of the *cysB* and *athA* loci, and of the distribution of allelic sites within these loci.

Clowes found that secondary biochemical differences in certain *cysB* mutants were manifested by the ability of four of the fourteen members of this group to grow slowly on hydrosulfite or sulfite. Three-point genetic tests carried out with tryptophan markers, to which all *cysB* strains have been shown to be linked, demonstrated that this heterogeneity is not confined to a localized zone, but that there are at least two discrete groupings of sites of mutations to the sulfite-utilizing phenotype, separated from each other and adjoined on both sides by sites of mutants having the originally defined phenotype. Thus, if the mutants able to utilize sulfite are designated *B'* and those unable to utilize this sulfur source are designated *B*, the *cysB* group has the following order: *B-B'-B-B'-B*. The close linkage among all the *cysB* mutants suggests that the size of this locus is of the same order as that of other single loci previously studied; and therefore it is unlikely that these five groupings represent five distinct but closely linked loci, particularly in view of the failure to differentiate phenotypically among any of the *B* mutants. Moreover, experiments show no abortive transduction between members of this group, al-

though that phenomenon is apparent in tests between members of *cysB* and other groups. The mutants do not behave as normal "leaky" mutants, since they show no growth on sulfate. It might be expected that accumulation of the intermediate shown as *X* in figure 1 would reduce its rate of production. If the enzyme-controlled reaction from  $S_2O_3^{--}$  is more sensitive to this accumulation than the autocatalytic reaction from  $S_2O_4^{--}$ , a "leaky" mutant at block *B* would be more likely to utilize the intermediates in the  $S_2O_4^{--}$  pathway than those in the  $S_2O_3^{--}$  pathway. The growth responses of the *B'* mutants might thus be due to such "leaky" blocks.

An interesting pattern of distribution of differentiated sites was observed by Ozeki at the *athA* locus. As was reported last year, pantothenate is able to satisfy to various degrees the thiamine part of the requirement in some adenine-thiamine mutants. All the *ath* mutants have been classified in three grades, according to their mode of growth on adenine-pantothenate medium: grade I, showing no growth; grade III, showing normal growth; and grade II, showing heterogeneous growth. The heterogeneous growth of the grade II mutants forms sectorial colonies on adenine-pantothenate medium, but transduction tests have failed to reveal any genetic difference between the bacteria forming a sector and bacteria of the original strain. The classification is as follows: *athA* grade I: -4, -18, -10 (multiple-site); *athA* II: -1, -2, -3, -7, -16; *athA* III: -8, -9; *athB* III: -6; *athC* I: -5, -14; *athD* I: -11, -12, -17; *athD* II: -15; *athD* III: -19, -20.

It seems likely that the "adenine-pantothenate" phenotype is intermediate between the prototrophic and adenine-thiamine phenotypes, and that it is due to incompleteness of the biochemical block in certain mutants, the various degrees of its expression being attributable to differences in degree of effectiveness of the biochemical blocks produced by mutations at different sites of a locus. This interpreta-

tion is supported by the following three lines of evidence: (1) grade III and grade II mutants have stronger residual growth on enriched minimal medium than grade I mutants; (2) suppressor mutations change the phenotype of grade I or II mutants to the grade III type; and (3) a similar, but temporary, modification of the phenotype of grade I mutants is produced by abortive transduction with the wild-type allele.

The phenotypic differences in nutritional requirements of various alleles of the *ath* loci make it possible to detect the donor type in transduction experiments between alleles showing these differences, and thus to determine their order on a genetic map on the basis of ratio of number of prototrophs to total number of donor type and prototrophs obtained. The genetic order of the *athA* alleles is as follows: 8(III)-7(II)-1,2,3(II)-4(I)-18(I)-16(II)-9(III). It seems that the distribution of sites within locus *athA* is not a random one; sites representing the typical adenine-thiamine phenotype (grade I) are in the central portion of the locus, whereas those representing incomplete biochemical blocks are located at the two ends. Thus our results suggest a polarity in the arrangement of sites, with a gradient from a stable center toward unstable ends. A similar distribution of sites is indicated for the *athD* locus.

All this evidence shows that mutants resulting from changes at different sites of a locus, although they are affected primarily in one function, may differ from one another in respect to various other properties. The findings indicate that the sites of alleles having similar properties are not distributed randomly within a locus, but tend to be grouped together.

#### MULTIPLE-SITE MUTANTS

The results of our first transduction experiments to determine the allelic relations among cystine-requiring mutants showed that *cys-20* failed to produce any

recombinants in transduction tests with six of the other cystine mutants, namely, *cys-1*, -3, -5, -13, -21, and -22, whereas recombinants were obtained in all tests of these six mutants with one another. The frequencies of recombination indicated that the six mutants are closely linked; and it was concluded that they are nonidentical alleles of one gene locus, which is now known as *cysA*. Subsequently, two more nonidentical alleles belonging to the *cysA* locus were isolated (*cysA-32*, -69), and neither of these produced recombinants with *cys-20*. Thus it is evident that *cys-20* is different from the other *cysA* alleles, in that it "covers" all the known sites of that locus.

Similar examples of multiple-site mutants are *hiB-22* and *athA-10*. The study of *hiB-22* made by Hartman showed that it covers all the known sites of the *hiB* locus, but does not include any of the sites of *hiA* or *hiC*, to the left and right of *hiB*. Yura's investigation of *athA-10* showed that it covers all the known sites of the *athA* locus.

An interesting situation with regard to the occurrence of multiple-site mutation has been analyzed by Clowes in the closely linked *cysC* and *cysD* loci. Mutants *cysC-43*, -47, -7, and -38 grow on either sulfite, hydrosulfite, or thiosulfate, and show reduced but significant frequencies of transduction with one another and with the mutants *cysD-23*, -51, -44, and -46. The last-named mutants utilize only hydrosulfite or thiosulfate, and show similarly reduced but significant transduction frequencies among themselves and with the *cysC* mutants. These particular *cysD* mutants feed all *cysC* and *cysA* mutants. Mutants *cysD-28*, -29, -37, -42, and -49, although resembling the other *cysD* mutants in their ability to utilize thiosulfate and hydrosulfite but not sulfite as alternative sulfur sources, give no evidence of transduction with each other or with any of the aforementioned *cysC* and *cysD* mutants except *cysC-43*; *cysD-36* gives no transduction with *cysC-7*, -38, or -47, or with



*cysD-51*. In experiments involving either wild-type donors or any other auxotrophic mutants, including those of other *cys* loci, however, these multiple-site mutants show the normal wild-type frequency of transduction. Moreover, they do not feed either *cysC* or *cysA* mutants and do not undergo reversion to the wild-type phenotype. The genetic evidence is consistent with the assumption that in *cysD-28*, *-29*, *-37*, *-42*, and *-49* mutants the mutation involves all known sites of the *cysD* locus as well as three of the four known sites of the *cysC* locus, and that the *cysD-36* mutation involves one site of the *cysD* locus and three sites of the *cysC* locus. Thus, among fourteen mutants associated with these two loci, six are multiple-site mutants, and five of these "cover" all the known sites of the *cysD* locus and most of the sites of the *cysC* locus. An analysis of fourteen additional mutants carried out by B. Djordjević showed that six of them are multiple-site mutants.

Neither *athA-10* nor any of the multiple-site *cysC-cysD* mutants reverts to prototrophy. Small-colony-forming "revertants" appear with a low frequency when *cysA-20* bacteria are grown on minimal plates. An analysis of these made by Sheila Howarth shows that they are due to mutations at a suppressor locus, rather than to back mutations at the *cysA* locus. Similar small-colony prototrophs were observed by Hartman in his work with the *hiB-22* mutants.

The known properties of multiple-site mutants, briefly summarized, are as follows: multiple-site mutants fail to produce recombinants in transduction tests with a number of closely linked markers; the frequency with which they are found at any one locus is, as a rule, lower than the frequency of occurrence of single-site mutants; they involve a well defined region of chromosome; they do not revert to prototrophy; and, in the one case (*hiB-22*) in which it is possible to study crossing over with adjacent markers, a multiple-site mutant exhibits a lower frequency of crossing

over than do single-site mutants of the same locus.

The failure to produce recombinants in tests with closely linked markers suggests that we are dealing with a mutation which extends over a certain length of the chromosome, and that either (*a*) crossing over leading to viable recombinants cannot occur at all within that length, or (*b*) if it does occur the recombinants cannot be recognized because they do not have the wild-type phenotype. The (*a*) condition could be produced by chromosomal aberrations such as deletions; the (*b*) condition, by simultaneous alterations at several adjacent sites.

When the accumulation of additional material allows more extensive study of multiple-site mutants, we may be able to discover what mechanism is responsible for their origin. The evidence available at present favors a deletion mechanism, and critical information is supplied by the data concerning the frequency with which multiple-site mutants are found, particularly the exceptionally high frequency of such mutants observed in the *cysC-cysD* region of the chromosome.

Our reasoning finds support in observations made with *Drosophila*, which can be briefly summarized as follows. Lethals are the most frequently observed mutations, whether spontaneous or induced by radiation or some other mutagen. Cytological studies of lethal mutations occurring in chromosome regions favorable for such study indicate that many of them are deletions, detectable in salivary-gland chromosomes. For example, our analysis in 1941 of thirteen spontaneous and forty-eight X-ray-induced Notch mutations showed that nine of the spontaneous and thirty-seven of the induced were deletions of various lengths, the spontaneous occurring within a region involving up to nine bands and the induced within a region involving up to fifty bands. Thus it was indicated that deletions are among the most frequently occurring of mutational events. Still earlier studies of lethals located in



various regions of the X chromosome showed that regions differ in their importance to the performance of physiological functions in a cell. Lethal mutations, presumably deletions, in most of the regions investigated were found to be lethal to even a few cells (cell lethal); lethals in a few regions were not cell lethal but were lethal to the organism as a whole; and, finally, a very few deletions were not lethal to the organism. If similar conditions obtain in bacteria, that is, if a considerable portion of genetic changes are deletions and if, because of haploidy, only those can be detected that occur in regions of the chromosome not essential for the functioning of the genome, then it is to be expected that deletions will be observed with a high frequency only in the rare nonessential regions. In our present material the *cysC-cysD* section of the chromosome would represent such a region. Rare survival of deletions in certain other regions—such as the *cysA*, *hiB*, and *athA* loci—could be explained by assuming that deletions in these regions have a strong detrimental, but not a lethal, effect on the functioning of the genome, and that, during the early divisions of cells carrying a deletion, mutation at some other locus compensates for the bad effect of the deletion.

In evaluating this proposed explanation it seems necessary to raise the question why, since we are dealing with nutritional mutants, addition of the required nutrient to the medium should not be able to compensate for a deletion, as it is able to compensate for a nondeletional genetic change. A possible answer is that a gene, in addition to controlling a specific function, also plays an important role in the general activity of the whole complex of genes—the genome—and that in this connection the absence of certain genes cannot be offset.

The results of our experiments could also be explained, however, by assuming that the deficiency created by a deletion can be wholly compensated for by the ad-

dition of appropriate nutrient to the medium, and that the high frequency of multiple-site mutations observed in the *cysC-cysD* region is caused by a high frequency of occurrence of the events responsible for these mutations. We hope by further experimentation to find new evidence for analysis of the responsible mechanism.

#### POPULATION DYNAMICS

The unique form of control of population growth in the chemostat (by a single and freely chosen nutrient factor) has enabled Moser to develop an exact theory of continuously growing populations of bacteria with a minimum of assumptions. The task was simplified by the exclusion of conditions that would allow the transfer of hereditary material from one cell to another in the population and thus create, through genetic recombination or dominance effects, a source of variability other than mutation. Since this study deals with populations of haploid though presumably multinucleate cells, the only basic genetic forces that need to be taken into account are mutation, phenotypic delay, and specific selection (differential cell reproduction).

The evolution of bacterial populations in the chemostat is regarded as a sequence of population changes; and it is assumed that each of these population changes is effectuated by a mutant variety (prevalent mutant variety) which, under conditions of growth limited by a single nutrient factor, displays a superior capacity to accumulate and therefore prevails over all other genetic variants (infrequent mutant varieties) present in the culture. Because of its predominance over all other genetic variants, the prevalent mutant variety controls the over-all dynamics of the bacterial population at each step of evolution in the chemostat. It may, through excessive accumulation, induce displacements from the position of growth equilibrium (steady state); that is, it may affect the concentration of controlling growth factor in the culture tube, the total-population density,

the growth rates, and the selection pressures. It may cause nonspecific or periodic selection against all other mutant varieties. Furthermore, it will determine the position of over-all genetic equilibrium.

On the basis of this population model a system of equations can be set up, formulating rates of change of cell densities, rate of change of concentration of controlling growth factor in the culture tube, and change of growth-rate constants as a function of limiting-nutrient concentration.

From these basic differential equations can be derived an explicit expression for the establishment of a constant cell density in the growth tube of the chemostat, as a function of time, for a bacterial population that remains fairly homogeneous. As a first approximation to this solution of the equations of growth, it is found that a homogeneous bacterial population grows logistically in the chemostat if the concentration of the controlling growth factor in the culture tube is much lower than the input concentration of controlling growth factor.

The steady state attained by a pure or fairly homogeneous population is not necessarily maintained over an unlimited period of time. If specific selection operates in favor of the prevalent mutant, its extensive accumulation, leading to a significant change in the composition of the population, induces systematic displacements from an initial growth equilibrium. Moser's theory furnishes explicit expressions for induced changes in total-population density, concentration of controlling growth factor in the culture tube, and growth-rate constants, as functions of the frequency of the prevalent mutant. By analysis of the effects of changing composition of the population on the growth-rate constants, it is possible to derive formulas for the accumulation of a prevalent mutant variety in populations that are initially fairly pure and in growth equilibrium. These formulas, in turn, enable one to establish in exact terms the criteria for the occurrence of population change-overs, and also to describe in mathe-

matical terms various types of change-over, such as, for example, the kind observed by Novick and Szilard in their chemostat experiments with a Tr-resistant, tryptophan-requiring B strain of *Escherichia coli*.

If the initial steady state of a homogeneous population is interrupted as the result of extensive accumulation of a prevalent mutant, growth equilibrium is re-established, although at a different position, when over-all genetic equilibrium is attained. This growth equilibrium (steady state of heterogeneous populations) maintains not only a constant total-population density and a constant proportion of bacteria belonging to the parent strain and to the prevalent mutant strain, but also constant densities of each of these strains.

The accumulation of mutants belonging to categories other than the prevalent mutant variety is controlled by mutation, by specific selection, and by nonspecific or periodic selection, and is therefore of a complex nature. The theory provides explicit expressions for the accumulation of such mutant varieties in a population which, at a certain time, changes over from the original strain to another, better "fitted" (e.g., faster growing) strain. These expressions predict various accumulation curves, which show the characteristic shifts or oscillations related to population change-overs.

In the final part of this population study, approximation functions are derived for the accumulation of infrequent mutant varieties in the chemostat; and these functions prove to be useful tools for curve fitting and for calculation of mutation rates and specific-selection pressures. On the basis of these simple accumulation functions, procedures are outlined for the estimation of low mutation rates and of specific-selection pressures from experimental accumulation curves. Statistical techniques for the adjustment of parameter values and for the calculation of the standard errors of these values are presented in detail. A practical example of curve fit-

ting indicates that a high degree of precision can be achieved in measuring bacterial mutation rates by means of the

chemostat technique, but that determinations of specific-selection pressures by the same method are a great deal less accurate.

## ORGANIZATION OF CELLULAR MATERIALS

B. P. KAUFMANN, M. R. McDONALD, H. GAY, K. E. FUSCALDO, D. N. DE, AND  
M. J. McELDERRY

During the past year, studies of the materials of heredity have been directed into three main channels of inquiry. The first concerns the fine structure of chromosomes; the second, the role of specific chromosomal loci in intracellular differentiation; and the third, the nature of the bonds responsible for maintenance of chromosomal integrity. Experimental details have been given in papers presented at two symposia, namely, the Arden House conference on fine structure in January and the Cold Spring Harbor Symposium on genetic mechanisms in June, so that the present report can be restricted to summarized statements of the essential findings and their significance.

The first two channels of inquiry listed above have involved use of the electron microscope. Micrographs were made with the RCA instrument at Brookhaven National Laboratory; we are indebted to Dr. Howard J. Curtis, Director of the Biology Department, for permission to use the microscope, and to Dr. Donald Fluke for his personal interest and for assistance in its operation.

Dr. Sibergina Wagenaar, of the Laboratory for Research in Plant Physiology of The Agricultural University, Netherlands, who held an award in the Research Scientists Program sponsored by the National Academy of Sciences-National Research Council, joined our group in February to undertake chemical and cytochemical studies of the changes effected in tissues of tobacco plants infected with mosaic virus. A brief statement of her findings is included in this report.

From time to time throughout the year we have had the benefit of temporary assistance. Workers who have served on this

basis include Sydney Smith, Suzanne Hume, Florence Powell, Sarah Terry, and Lois Glass. Their devoted attention to details of the program has greatly facilitated the work of the regular staff members.

Our research efforts have again been furthered by a grant (RG-149) from the National Institutes of Health, U. S. Public Health Service.

## PATTERNS OF CHROMOSOME ORGANIZATION

Continuing progress in the efforts of geneticists to translate the symbolism of gene and mutant into the more precise terminology of cell dynamics requires a more profound understanding than we now have of the patterns of organization of chromosomal materials. With this objective before us, we have continued our studies of somatic and meiotic chromosomes at the level of resolution afforded by the electron microscope. Our choice of materials has been determined by the desire to integrate interpretations of fine structural detail with the broad foundation of observational and experimental evidence developed during the past quarter of a century. Chromosomes of the salivary glands of *Drosophila* and of monocotyledonous plants such as *Tradescantia* have been studied most extensively with the light microscope and were therefore chosen for further examination with the electron microscope.

*The salivary-gland chromosomes of D. melanogaster.* The more general aspects of the structure of these chromosomes, as determined by Gay, were reported in Year Book No. 54. There is no limiting membrane to the chromosome, which consists of a large number of coiled chromonemata, assumedly disposed in intertwined pairs



to form a hierarchy of pairs of pairs. The chromonemata are coiled throughout their length, within banded as well as interband regions. Differences in the stainability of these two kinds of region with the Feulgen method thus appear to depend in part on differences in the pattern of coiling of the threads and in part on differences in the amount of deoxyribonucleic acid (DNA) per unit length.

Gay and Fuscaldo have now extended these observations by determining the effects of nucleases and proteases on the disposition and electron-scattering properties of the constituent chromonemata. The buffered osmium tetroxide fixative used so extensively for electron microscopy makes tissues more resistant to enzymatic hydrolysis than the acetic-alcohol fixative used in our earlier cytochemical studies. Our previous experience with fixatives containing osmium (e.g., Flemming's fluid) had shown, however, that high concentrations of enzyme can hydrolyze materials preserved in these fluids. Since the methacrylate imbedding medium also seems to have an inhibitory effect on enzymatic action, a treatment procedure was developed which involves digestion of the intact fixed salivary gland in high concentrations of enzyme and subsequent imbedding of the tissue. Comparison of electron micrographs of thin sections of the treated gland with light-microscope photographs of thick sections of adjoining tissue served to locate the chromosomes and to determine the effectiveness of the enzymatic hydrolysis. The other gland of the pair from the same individual was used as a control. By these methods it was found that hydrolysis with deoxyribonuclease removed material from both band and interband regions of chromosomes, as was evidenced by reduction in density in the electron micrographs. Treatment with ribonuclease produced no appreciable loss of density in the chromosomes but caused some reduction in the nucleolus. The fact that the nucleolus sometimes showed reduction in localized areas after deoxyribonuclease treatment is

regarded as evidence of localization of the associated nucleolus-organizing chromosomal strands. Trypsin in buffer caused a loosening of the strands of the chromosome but not their dissolution, and revealed with greater clarity than in the control sections the ultimate chromonemal fibers. These cytological and cytochemical studies are being extended to determine the changes effected in patterns of chromosome organization by different fixatives.

*Chromosomes in dividing cells of Tradescantia.* The structure of the chromosomes during the prophase stages of somatic and first meiotic mitoses has been studied by Kaufmann and De. In both types of cells the chromosomes appear to be polynemic, or composed of a number of fibrillar elements.

The most striking micrographs were those of very early prophase chromosomes of staminate-hair cells. In these cells, as in those of the salivary gland of *Drosophila*, the pattern of organization of the chromosomes seems to reside in a hierarchy of pairs of pairs of helically disposed chromonemata. When seen in end view, each set of pairs appears to constitute a rim of densely electron-scattering material surrounding a less dense cylindrical "core." At the lowest order of reliably interpretable detail the rim appears to be about 40 Å wide surrounding a 40-Å central axis. If these dimensions encompass a pair of chromonemata, as we have assumed, they cannot be far different from those of the ultimate nucleoprotein fibrillar elements. Comparison of the dimensions of the finest discernible strands with those of the entire chromosome suggests that the somatic prophase chromosome of *Tradescantia* is compounded of at least 64 (and possibly 128) subsidiary units. But whether the members of each pair are intertwined (a plectonemic relationship) or lie side by side without interlocking (a paranemic association) could not be determined with accuracy, except in the highest order, where the sister chromatids are obviously intertwined in the classical pattern of rela-



tional coiling. These problems, as well as questions relating to the disposition of nucleic acids and proteins in the subsidiary units of the chromosome, will eventually be resolved, we anticipate, through the application of such methods of cytochemical analysis as we have developed in our earlier studies.

From the foregoing considerations it appears probable that the chromosomes of the dividing plant cell and those of the salivary gland are not essentially different in basic structural pattern. There are, of course, major differences as a consequence of the method of origination of the salivary-gland type, which involves the pairing of homologues and differential growth in the transition from the mitotic-type progenitor. A full consideration of these differences would lead into questions of patterns of coiling—not only those initially produced but also those secondarily derived—which cannot be answered satisfactorily until a more adequate mass of observational data about fine structure has been accumulated. Work along these lines is now in progress.

*Structural multiplicity and functional unity.* Accepting the interpretation that a dividing chromosome contains a large number of constituent fibrillar units (and, it may be added, there is a considerable amount of experimental as well as observational evidence in support thereof), it becomes necessary to re-examine in terms of basic structural units such genetically significant phenomena as mutation, crossing over, and related types of structural rearrangement, and to consider the adequacy of the models that have been proposed to explain such phenomena. In doing so we must determine the nature of the materials or conditions that mold a bundle of strands into an aggregate which acts as a unit. In other words, the concept of a multiplicity of subordinate units must be correlated with the vast store of evidence suggesting that gene mutations and structural rearrangements usually affect whole chromosomes or chromatids (although

mosaics are by no means uncommon), that crossing over occurs at a "four-strand" stage, that the number of centromeres equals the number of chromatids, and so on. The questions that arise are complex, and no definitive answers can be offered. In approaching the problem experimentally we have initiated electron-microscope studies of the spermatozoa of *D. melanogaster*, since treatment of these cells yielded a large share of the evidence on which our present concepts of genetic mechanisms are based.

#### SPERMIOGENESIS IN *DROSOPHILA*

During the summer of 1955, Mr. John F. Aronson began in this laboratory an electron-microscope study of spermiogenesis, or transformation of the spermatid into the spermatozoon, in *D. melanogaster*. Examination of the micrographs obtained in these preliminary studies affords some hitherto unreported information about patterns of structural organization.

The photographs suggest that the dissociation of sister spermatids is effected by a process involving constriction, associated with loss of cytoplasm through pinching off or blebbing. As the spermatid develops and elongates, the nebenkern (of mitochondrial origin) assumes the form of paired ribbon-shaped elements lying in proximity to the axial filament but without any identifiable pattern of internal organization.

One of the key problems involved in a study of this type, and the one that prompted these investigations, concerns the nature and distribution of the chromosomes in the sperm head. Unfortunately, we cannot yet report much progress in analysis of the configurational patterns within the sperm nucleus. One set of photographs does suggest that the chromosomes may be arranged linearly end to end, but much more observation and experimentation (including treatment with enzymes for localization of specific materials) will be needed to determine the validity of this preliminary interpretation.

The pattern of organization of the flagellum, or sperm tail, on the other hand, is particularly well defined in the micrographs and merits brief description. It has been known for many years from studies with the light microscope that the flagella of spermatozoa of many species are composed of subsidiary fibrils. More recent studies with the electron microscope have revealed the presence of nine to twelve longitudinal fibrils; and M. L. Watson's study of thin sections of the sperm tail of the rat, for example, has shown that the strands are distributed in a precise pattern, with two in the center and nine peripherally arranged. This pattern agrees with that observed by D. W. Fawcett and K. R. Porter in cross sections of cilia of epithelial cells of molluscs, amphibians, and mammals. These workers found that each of the nine peripheral filaments was double, whereas the two central ones were single. Transverse sections of the flagellum of *D. melanogaster* obtained by Aronson also reveal the basic pattern of nine peripheral and two central fibers, but show, in contrast with the above-mentioned observations, that each of the nine peripheral filaments is composed of four or five radially disposed subsidiary fibrillae. The cross-sectional view of the flagellum shows in addition to these pinwheel-like configurations, which are marginally disposed, a more or less homogeneous, densely electron-scattering, centrally placed structure. This filament appears to extend for most of the length of the tail.

#### CHROMOSOMAL CONTROL OF CELLULAR DIFFERENTIATION

During the past year, Gay has extended her observations on nucleocytoplasmic relations in *Drosophila* salivary-gland cells (Year Book No. 54). Nuclear-membrane blebbing had previously been studied only in cells at one stage of development, during mid-third instar, and it was necessary to determine whether this phenomenon occurs throughout the life of the salivary-

gland cell. Since nuclear blebbing had been proposed as a mechanism for genetic control of cytoplasmic function, it was also considered pertinent to determine whether the appearance of nuclear-membrane out-pocketings is directly related to cellular function.

The salivary glands of the larval *Drosophila*, as their name implies, are concerned with producing digestive fluids. G. Fraenkel and V. J. Brookes have recently indicated, however, that during the third instar these glands assume a second secretory function, the formation of a puparial glue. Although these authors made no cytological or cytochemical determinations, they suggested that certain cytoplasmic globules which had been noted in third-instar cells by other cytologists were the forerunners of the puparial glue.

The proposal of a dual secretory function in third-instar salivary glands suggested that the nuclear-membrane out-pocketings observed by Gay in the mid-third-instar cells might be a manifestation of a change in cellular activity. In order to trace the cellular changes that occur during development, a combined light-microscope and electron-microscope study was made of salivary-gland cells of *D. melanogaster* during seven stages of larval development selected at regular time intervals from the end of the second instar to pupation. Cytochemical tests showed that the granules of the third-instar cells were composed of a mucoprotein. Cells of the early third instar had no secretion granules, and the cytoplasm of the prepupal cells contained only a few, but the lumen of the prepupal salivary gland was filled with a secretion that gave the same cytochemical reactions as the granules, indicating that the secretion had been discharged from the cells.

Electron-microscope observations of the same glands that were used for light microscopy and for cytochemical tests indicated that during the early stages of development nuclear-membrane blebbing was entirely absent, but that it appeared at

about the same time as the secretion granules, that is, when one-third of the third instar had been completed, and persisted throughout the remainder of this instar. Just before puparium formation, however, fewer blebs were found. The events revealed by combined light-microscope and electron-microscope study suggested a correlation between the appearance of nuclear-membrane outpocketings and the production of secretion granules.

These observations indicate that the structural relations in mid-third-instar salivary-gland cells reflect the functional activity of chromosomes in producing materials which subsequently are contributed to the cytoplasm. Cytochemical studies utilizing enzymatic hydrolysis and electron microscopy suggest that the nuclear-membrane blebs contain DNA, and that the highly differentiated chromosomal material always associated with the outpocketings is partly composed of RNA. A more thorough investigation, using other enzymes and tests, must be undertaken before these results can be considered definitive.

The findings summarized above provide added evidence that nucleocytoplasmic exchanges in *Drosophila* afford a mechanism for chromosomal control of cellular differentiation. Operation of the mechanism involves an intimate association between some chromosomal loci (perhaps heterochromatic) and the nuclear membrane, which produces blebs in these regions. The blebs become detached into the cytoplasm and are converted into ergastoplasmic membranes, while their contents augment the store of cytoplasmic material. This mechanism might represent a unique adaptation, but during the past year there have been several reports implicating the nuclear membrane in the formation of cytoplasmic-membrane systems in other types of cells. Attention is consequently focused on the nuclear membrane as an actively functioning component of the cell. On this basis, Kaufmann and Gay have considered the genetic implications of the

nuclear membrane-blebbing phenomenon. They have suggested that in the salivary-gland cell the operation of heterochromatin in protein synthesis may be mediated through this mechanism. The increased basophilia observed near the nucleus by T. Caspersson and J. Schultz, and others, may be due to formation of basophilic ergastoplasmic lamellae, which originate from nuclear-membrane blebs that are probably associated with heterochromatic regions of the chromosomes. This hypothesis offers a more precise definition of the role of heterochromatic loci other than the nucleolus-organizing region, and recognizes an active rather than a passive participation of the nuclear membrane. The question is raised whether a more specific activity of heterochromatin, such as an effect on adjacent euchromatic loci leading to variegation, might be attributable to the operation of a mechanism analogous to that described above.

If a specificity afforded by adjacent chromosomal material could be transferred to the nuclear membrane either during its formation or subsequently, detachment of nuclear-membrane segments could represent a method for production of "plasmagenes." Transfer of representatives of nuclear genes to the cytoplasm is the primary criterion on which the concept of the plasmagene is based, in conjunction with the assumption that they can then divide and mutate in the cytoplasm. On the basis of evidence now available in *Drosophila*, it is not known whether the ergastoplasmic lamellae produced by nuclear-membrane blebs are capable of either duplicating or mutating. H. Swift and L. I. Rebhun have suggested that in some oöcytes the ergastoplasmic lamellae may form new lamellae on the free margins of the basophilic lamellar aggregates. This would constitute evidence of replication.

The hypotheses discussed above involve intracellular transfer of specificity. The question might be posed whether intercellular transport of specificity might likewise be effected, by transfer of similar



cytoplasmic organelles from one cell to another.

The problems of gene action raised in this discussion are amenable to an experimental and observational approach, and it is hoped that future electron-microscope studies may further elucidate them.

#### MAINTENANCE OF CHROMOSOMAL INTEGRITY

Within the past two years a number of investigators have attempted to evaluate the role of divalent cations in maintenance of chromosomal integrity. The investigations stem largely from observations reported by D. Mazia of the effects of the chelating agent ethylenediaminetetraacetic acid (EDTA) on salivary-gland chromosomes of *Drosophila* and the mitotic and meiotic cells of grasshopper testes. The changes that occurred were interpreted as indicating degradational action of EDTA through removal of bonding calcium and magnesium ions.

Since we had reached a different interpretation of the mode of action of EDTA in preliminary and unpublished analyses made in connection with studies of the action of trypsin and pepsin, it seemed desirable to re-examine the problem in greater detail. This has been done in the past year, through chemical and cytochemical analyses of the action of the commercial preparation Versene on cellular materials (by Kaufmann and McDonald) and through genetical analysis of the effect of this agent on crossing over in *Drosophila* (by Kaufmann, Gay, and McElderry, with considerable assistance from Fuscaldo and Powell).

*Effects of Versene on cellular materials.* When used on salivary-gland cells of *Drosophila* that have been fixed by freezing, a 0.1 M solution of Versene in the pH range 6 to 7 preserves the chromosomes with a degree of fidelity approaching that obtained with 45 per cent acetic acid, a 0.01 M solution produces some deformation, and a 0.001 M solution causes enlargement of the nucleoli and peripheral

displacement of the chromosomes. If extensive rinsing in water follows treatment with the 0.001 M concentration, structural deformation is less marked than in the absence of the water rinse. Moreover, if treatment with 0.001 M Versene is continued for 24 to 48 hours, there is less structural deformation than after shorter periods of treatment (4 to 6 hours).

These findings are in some respects similar to those obtained in our studies of the action of trypsin and pepsin, and the possibility accordingly emerges that Versene also effects degradation primarily through changes in the gel-like properties of cellular nucleoproteins. To test this possibility, salivary-gland cells were treated with ribonuclease before exposure to the action of Versene, and it was then found that the digested cells were not so sensitive to the action of 0.001 M Versene as the controls.

Another way of demonstrating that changes in the gel-like properties of cellular materials are involved is to expose the cells to the action of 0.1 M Versene and then to rinse them in water. A marked swelling rapidly occurs, which can be reversed by replacing the water with 0.1 M Versene; and this process can be repeated time and again, with gradual loss of cellular materials and structural deformation of the chromosomes. The swelling action is not inhibited by the use of 0.002 M  $\text{CaCl}_2$  or  $\text{MgCl}_2$ .

The foregoing results, when viewed in the light of the trypsin-pepsin studies, suggest that ribonucleic acid (RNA) is involved in these responses. Further studies showed that 0.001 M Versene actually effects some modification of stainability of cellular materials with dyes that serve to localize basophilic materials. It was also found that this chelating agent can produce the same types of mitotic abnormalities in living root-tip cells of onion as are induced by ribonuclease (see Year Book No. 52).

Chemical analyses were also made of the nucleic acid, phospholipid, and protein content of homogenates of root tips of onion



TABLE 1

THE EFFECT OF VERCENE ON THE SPONTANEOUS DEGRADATION OF VARIOUS COMPONENTS OF  
ONION-ROOT-TIP HOMOGENATES AT 3° C, pH 7

TREATMENT	CONSTITUENT MEASURED (EXPRESSED AS PER CENT OF THE ORIGINAL CONCENTRATION)			
	Protein	Phospholipid	DNA	RNA
H <sub>2</sub> O for 18 hours.....	96	72	79	86
H <sub>2</sub> O for 50 hours.....	89	48	60	80
0.001 M Versene for 18 hours.....	94	60	78	70
0.001 M Versene for 50 hours.....	89	50	79	56
0.01 M Versene for 18 hours.....	91	79	94	32
0.01 M Versene for 50 hours.....	89	60	96	25
0.1 M Versene for 18 hours.....	97	90	97	90
0.1 M Versene for 50 hours.....	94	76	101	78

that had been frozen in water before homogenization and then left at 3° C in various concentrations of Versene at pH 7. The results, which were based upon analysis of aliquots removed at several time intervals, indicate that the chelating agent did not alter the amount of protein, and that the spontaneous degradation of phospholipid and of DNA was inhibited by concentrations of Versene greater than 0.001 M, whereas that of RNA was markedly accelerated by 0.01 M, less so by 0.001 M, and not at all by 0.1 M (table 1).

All the experimental findings and their manifold implications cannot be presented

in detail here, but they suggest that Versene has a pronounced effect on cellular RNA. The effect is not necessarily a direct one, but probably manifests itself through interference with cell metabolism, as will be emphasized later.

*Effect of Versene on crossing over.* The experiments mentioned above indicated the desirability of testing the effect on crossing over of an agent capable of degrading RNA in the living cell, namely ribonuclease, and comparing its action with that of Versene. The results of such an experiment, using the white, miniature, and forked markers in *D. melanogaster*, are shown in table 2. An extensive statistical

TABLE 2

EFFECT OF EDTA ON CROSSING OVER IN THE WHITE-MINIATURE-FORKED REGION OF THE X  
CHROMOSOME OF *Drosophila melanogaster*

Treatment	Noncrossover	Crossover (w-m)	Crossover (m-f)	Crossover (w-m-f)	Totals
Dry (not immersed).....	10,967	4,503	2,814	494	18,778
RNase 0.00007 M solution.....	13,168	5,854	3,731	676	23,429
EDTA 0.01 M solution.....	15,861	6,657	4,202	710	27,430
Water (distilled).....	17,740	7,753	4,636	804	30,933
Totals.....	57,736	24,767	15,383	2,684	100,570
%.....	(57.41)	(24.63)	(15.30)	(2.67)	(100.01)
	$\chi^2$	P		$\chi^2$	P
RNase vs. dry	21.46	ca. 0.0001	Dry vs. water	7.88	ca. 0.05
RNase vs. EDTA	15.46	ca. 0.0015	Dry vs. EDTA	1.94	ca. 0.6
RNase vs. water	14.74	ca. 0.0020	Water vs. EDTA	5.35	ca. 0.15

analysis of the total data showed that ribonuclease significantly modified the frequency of crossing over in the intervals tested as compared with Versene and the controls. The control flies were obtained from either water-treated or untreated larvae (the latter designated by the word "dry"). The results are not attributable to differential survival of the various types of progeny obtained in the different types of treatment.

Other experiments, involving different methods of treatment, indicated that under certain conditions Versene may be as effective as ribonuclease, or even more effective, in modifying crossing over. We are thus led to the conclusion that any agent that alters the normal metabolism of the cell, whether by changing the ionic environment or by removing necessary cell constituents such as RNA, may cause instability that is reflected in modification of chromosome form and behavior. The results outlined above cannot readily be brought into accordance with the assumption that chelating agents exert their effects on the chromosomal fabric solely by the removal of calcium and magnesium ions, which serve as bridges between the macromolecular complexes of nucleic acid and protein of which the chromosomes are composed.

#### STUDIES OF TOBACCO MOSAIC VIRUS INFECTION

Wagenaar has continued to study the effect of tobacco mosaic virus infection on developing tobacco leaves. Her earlier investigations, carried out in the laboratories of Dr. B. Commoner at Washington University, had shown that leaves infected during development exhibit the typical, well known, light-and-dark-green mosaic pattern. The dark-green color is similar to that of healthy plants. In some leaves it is found around the secondary veins; in others, no regularity in pattern can be observed. Using a salt extraction procedure developed by Dr. Wang at Washington

University, she had found the total nucleic acid and protein content of similar areas of infected light- and dark-green tissues to be approximately equivalent, but had noted marked differences in the DNA content. The dark-green areas had two to three times as much DNA as the light-green ones; and there was a similar disparity with regard to the proteins associated with the DNA fraction. The DNA content of the dark-green areas was slightly less than that of healthy young leaves, whereas the content of the light-green parts was about the same as that of leaves which were infected after they had finished development and therefore had no mosaic pattern. The light-green parts had about three times as much virus as the dark ones; their virus content was about the same as that of the older infected leaves. Similar results were obtained in this laboratory when the same salt extraction procedure was followed.

In her more recent studies Wagenaar used Schneider's trichloroacetic acid fractionation procedure. Again, the total nucleic acid content of infected and noninfected tissues was found to be the same, but this time no differences were noted in DNA content. Since several days are required in the salt extraction procedure for the DNA fraction to be obtained, the differences in findings by the two procedures might have been due to changes occurring in the homogenates during the salt fractionation. Homogenates were therefore left for several days at 2° C, and aliquots were analyzed by Schneider's method at several time intervals. No significant differences in total nucleic acid or DNA content of either healthy or infected tissues were found, even after five days. The reasons for the discrepancies in the results obtained by the two methods are thus still obscure, and more experimental data will have to be accumulated before any definite decision can be reached about the effect of infection with tobacco mosaic virus on the DNA content of tobacco leaves.

## MUTATION IN MAIZE

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Studies of controlling elements in maize were continued during the past year, with the purpose of learning more about the ways in which they control gene action and the types of action induced. Some of the results of these studies will be reviewed here. In addition, an investigation was begun of a particular alteration in structural organization of one chromosome of the maize complement, which has been found to induce still other changes, not only in chromosome organization but also in gene expression. A review of this case will be included.

*Ac* CONTROL OF MUTATION AT THE BRONZE LOCUS IN CHROMOSOME 9

The insertion of the controlling element *Ac* at the bronze locus in chromosome 9, and its effects on gene action at that locus, were mentioned in Year Book No. 54. Study of this case was continued in order to extend the evidence that mutation is related to removal of *Ac* from the locus or to other events induced by *Ac*. The phenotype appearing when *Ac* resides at or close to the bronze locus is similar to that produced by the standard recessive, *bz*. Mutations occur, and give rise either to a *Bz* type of expression of genic materials at the locus or to a stable recessive expression similar to that given by the standard recessive, *bz*. Mutations leading to a stable recessive expression occur more frequently than those producing a *Bz*-type expression, as is made apparent in the frequency of appearance of germinal mutations when plants that are homozygous for the bronze locus with *Ac* are used as females in crosses with plants that are homozygous for the standard *bz* allele, which is stable in the presence of *Ac*. Eleven such plants were used as female parents in this type of cross; and among the total of 2552 kernels that appeared on the ears they produced, 2398 exhibited spots of *Bz* in a recessive bronze back-

ground—the type of expression that appears when *Ac* is present at the bronze locus in the initial endosperm nucleus of the kernel. In addition, 28 kernels were totally *Bz* in phenotype, and 126 were totally bronze, with no spots of the *Bz* phenotype. Mutation to a stable *bz* expression is thus four and a half times more frequent than mutation to *Bz*. This same ratio for the two main types of germinal mutation also appears on ears of plants that are heterozygous for the bronze locus with *Ac*. Plants used in tests to identify these mutations were also made heterozygous for two additional genetic markers located to either side of the bronze locus. These plants were used as female parents in crosses with plants that were homozygous for the standard recessive alleles. Table 3 shows the types of kernels that appeared on the ears of the tested plants. A double crossover involving the regions *sh* to *bz* and *bz* to *wx* is rare. Therefore, the numbers of totally *Bz* and totally *bz* kernels shown in the *Sh Wx* class of section A of the table and in the *sh wx* class of section B represent, on the whole, the numbers of germinal mutations that occurred at the bronze locus with *Ac* inserted. It will be seen that the ratio of the two types of germinal mutation is the same as that produced by the homozygotes.

In order to determine the part played by *Ac* in the mutation process, plants were grown from some of the *Bz* kernels and also from some of the totally *bz* kernels in the *Sh Wx* noncrossover class of section A of table 3. All the plants that gave rise to these kernels had been tested for *Ac* number and location; and selections of kernels showing germinal mutations were made only from plants having one *Ac*, residing at the mutable bronze locus. Plants were grown from 16 kernels that exhibited the *Bz* phenotype. Each was tested for presence or absence of *Ac*, for its location if present, for transmission of the



chromosome carrying the *Bz* mutation through pollen and egg, for crossover relations with the linked markers, for stability of the *Bz* mutation in the presence of *Ac*, and for viability of the homozygote. Of the sixteen *Bz* mutants, fourteen proved to be stable. In six of the fourteen plants that had such a stable *Bz* mutation, *Ac* was absent. In five plants, one *Ac* was present but it was no longer located close to *Bz*: in four cases it was not linked with markers carried in chromosome 9, and in one case it was located close to *Wx*. In the

The *Bz* mutation in two of the plants derived from the 16 selected kernels that showed a *Bz* phenotype proved to be unstable. In both plants, *Ac* was present and located close to the locus of *Bz*. It could be shown that the mutations which subsequently occurred were instigated by this *Ac* element. Some of them effected stability of *Bz* expression. Three cases of stable *Bz* expression were examined, and in all three the stability was found to be associated with removal of *Ac* from the vicinity of the *Bz* locus. Other mutations

TABLE 3

PHENOTYPES OF KERNELS APPEARING ON EARS PRODUCED BY PLANTS HETEROZYGOUS FOR THE BRONZE LOCUS WITH *Ac*, AND FOR GENETIC MARKERS LOCATED TO EITHER SIDE OF THIS LOCUS, WHEN CROSSED BY PLANTS HOMOZYGOUS FOR THE RECESSIVE ALLELES

A. ♀ *Sh bz*<sup>Ac</sup> *Wx/sh bz wx* × ♂ *sh bz wx/sh bz wx*

B. ♀ *sh bz*<sup>Ac</sup> *wx/Sh bz Wx* × ♂ *sh bz wx/sh bz wx*

PHENOTYPE OF KERNEL WITH RESPECT TO ALLELES OF <i>Sh</i> AND <i>Wx</i>	A PHENOTYPE OF KERNEL WITH RESPECT TO BRONZE			B PHENOTYPE OF KERNEL WITH RESPECT TO BRONZE		
	Spots of <i>Bz</i> in bronze background	Totally <i>Bz</i>	Totally <i>bz</i>	Spots of <i>Bz</i> in bronze background	Totally <i>Bz</i>	Totally <i>bz</i>
<i>Sh Wx</i> .....	2457	41	158	1	4	927
<i>sh wx</i> .....	0	1	2596	881	12	56
<i>Sh wx</i> .....	641	3	87	35	0	230
<i>sh Wx</i> .....	49	0	671	202	0	58

three remaining plants, one *Ac* was present and it was located close to but probably to the right of *Bz*. Thus, in eleven of these fourteen cases of mutation to a stable *Bz* allele, it could be shown that the mutation-producing event was associated with removal of *Ac* from the bronze locus. In five cases, it was transposed to a new location. It may well be that such transpositions of *Ac* were also responsible for the origin of the *Bz* mutant in the six cases in which *Ac* was absent in the plant derived from the *Bz* kernel. If the transposition had occurred in a premeiotic cell, segregation in the subsequent meiotic mitoses of the chromosome carrying *Ac* in its new location could have produced a gamete carrying the *Bz* mutation but lacking *Ac*.

effected return to the unstable recessive, and in the eight cases that were examined *Ac* was present and was responsible for the instability. Still others gave rise to stable recessives, but none of these was examined for the presence or absence of *Ac*. Mutations of other kinds were also noted; but only one, a very rare type, will be mentioned here. This mutation caused a marked reduction in anthocyanin pigmentation. The event responsible for it was associated with removal of *Ac* from the *Bz* locus and its insertion elsewhere. It proved to be stable only in the absence of *Ac*; if *Ac* was present somewhere in the chromosome complement, mutations occurred to produce alleles having either a higher or a lower level of anthocyanin pro-



duction. They represented a change from apparently direct control of mutation by *Ac* to the "*Ds-Ac*" type of control. Two other cases of change to this type of control of the mutation process were found, and these will be discussed shortly.

Twenty-four cases of what appeared to be mutation to a stable recessive (*bz*) expression were also examined. Three of them occurred early in plant development, in three different plants. Each was made evident by the appearance, on one ear of the plant, of a sector in which all the kernels were totally bronze in phenotype; that is, none of the kernels within the sector exhibited any *Bz* spots. Plants were grown from some of the *Sh* and *sh* kernels in each of these three sectors, and were tested for the presence or absence of *Ac*, and for its location if present. Plants derived from the *Sh* class of kernels were also tested for stability of the *bz* mutant in the presence of *Ac*, for transmission through pollen and egg of the chromosome carrying the mutant, and for viability of the mutant when homozygous. From these tests it was learned that in each of the three cases a mutation to stable *bz* expression, occurring in a somatic cell early in plant development, had led to the appearance of the sector. Chromosomes carrying the mutations were normally transmitted through pollen and egg, and the homozygotes were viable. The mutation process was associated in all three cases with removal of *Ac* from the bronze locus. None of twelve plants derived from 7 *Sh* and 5 *sh* kernels from one of these sectors had *Ac*, although it was known that *Ac* was present at the bronze locus in the *Sh*-carrying chromosome in other kernels on this ear. In the other two cases, it could be determined with certainty that the mutation to the stable recessive was associated with transposition of *Ac* to a new location. In twenty-one plants (twelve derived from *Sh* kernels and nine derived from *sh* kernels), one *Ac* was found to be present. In the remaining fourteen plants (eight derived from *Sh* kernels and six derived from

*sh* kernels), no *Ac* was present. In those plants having *Ac* that were also heterozygous for markers carried in chromosome 9, no linkage of *Ac* with these markers was evident.

Early-occurring transposition of *Ac* is relatively rare, so that sectors of the type described above are not frequently observed. Usually, transposition of *Ac* occurs relatively late in the development of plant tissues, including the sporogenous tissue. Germinal mutations are recognized in single kernels distributed more or less randomly on an ear. Twenty-one of the twenty-four examined cases of mutation to stable *bz* were derived from kernels of this type, in the *Sh Wx* class, which exhibited no spots of *Bz* but appeared to be completely bronze in phenotype. Plants grown from them were tested for the presence or absence of *Ac*, for its location if present, for stability of the bronze expression in the presence of *Ac*, for transmission of the mutant through pollen and egg, and for viability of the mutant when homozygous. In all twenty-one cases, the mutation was normally transmitted through pollen and egg and the homozygotes were viable. In eight of the twenty-one plants, no *Ac* was present; and in all eight cases the *bz* expression proved to be stable when *Ac* was introduced into a nucleus carrying the *bz* mutant. In three other plants, one *Ac* was present but it was not linked with markers in chromosome 9; these *bz* mutants also were stable. In another three plants, one *Ac* was present, and was located in chromosome 9—close to *Wx* in one plant, close to *Sh* in another, and close to *bz* in the third. In these three cases also the *bz* mutant proved to be stable. In six other plants one *Ac* was present and was carried in chromosome 9, but its exact location within the chromosome was not determined. Recombination with *Wx* ranged from 20 to 35 per cent. It is suspected that *Ac* may have been located close to the *bz* locus in at least two of these six cases, for an occasional kernel carrying the *bz* mutant ex-

hibited a small *Bz* spot. These two cases may represent extreme examples of change in rate of mutation to *Bz* associated with a change in *Ac* that does not effect its removal from the vicinity of the locus. Changes of this type are known to occur. In the remaining plant of the twenty-one, two *Ac* elements were present, one located close to *bz* and one not linked with markers in chromosome 9.

It is clear that, in at least sixteen of the twenty-four cases described, mutation to a stable recessive was associated with removal of *Ac* from the locus of bronze, and that in seven of them *Ac* was transposed to a new location.

Another type of change may accompany removal of *Ac*. It brings about substitution of the *Ds-Ac* system of control of mutation at the bronze locus for the apparently direct control of this process by *Ac*. After such a change occurs, it can be shown that a *Ds*-type element, instead of the *Ac* element, resides at the bronze locus, and that the response of this *Ds* element to *Ac* brings about the observed mutations. In the absence of *Ac*, no mutations occur. A case of this type was mentioned earlier; and two additional cases have been detected. Both of these exhibit a stable *bz* expression in the absence of *Ac*, but undergo mutations to higher alleles of *Bz* when *Ac* is present. The mode of origin of the change in type of control of the mutation process is not yet understood. It is conceivable, however, that in the original case both a *Ds* and an *Ac* element are present, located close together, and that the apparently direct *Ac* control of the mutation process is deceptive because of frequent simultaneous removals of both elements from the vicinity of the bronze locus when a mutation occurs. Another possibility is that a *Ds* element may be substituted for an *Ac* element at the time of removal of *Ac*. This substitution is readily conceivable, and could equally well account for the appearance of a *Ds*-type element at the bronze locus in the three cases mentioned above.

#### CONTROL OF GENE ACTION BY A NON-TRANSPOSING *Ds* ELEMENT

The effects produced by *Ds* on the action of genic materials located to either side of it, after its insertion just distal to *Sh*, were reviewed earlier (Year Books Nos. 51, 52, and 53). It induces mutations in genic substances located to its right, which affect *Sh* or both *Sh* and *Bz* simultaneously. It also induces mutation of genic substances located to its left, including the locus of *I*. Some of the mutants so produced are unstable and undergo reversions. It can be shown that the *Ds* element is also responsible for these reversions, but in none of the cases examined was *Ds* altered in location when such a reversion occurred. The *Ds* element, when inserted at some other position, is known to be readily transposable to new locations. Such transpositions are usually associated with mutation of genic substances at the locus where *Ds* has been residing. Particular attention was given, therefore, to investigating those changes in gene action induced by *Ds*, when located just to the left of *Sh*, that were readily reversible. This was done in order to determine whether the apparent fixity in position of *Ds*, after insertion just to the left of *Sh*, would be maintained in every case, as the previous evidence suggested. One such case, considered to be particularly suitable for the purpose, was examined, and the results will be reported below.

As was mentioned above, some of the *Ds*-induced modifications effected a change in expression of both *Sh* and *Bz* and resulted in the appearance of the double mutant, *sh bz*. In the case to be described, the *bz* component but not the *sh* component proved to be mutable, and mutations to higher alleles of *Bz* occurred. It was detected in the following manner. A plant homozygous for *I*, *Ds*, *Sh*, and *Bz*, and also carrying one *Ac*, was used as female parent in a cross with a plant that was homozygous for *C*, *sh*, and *bz* and had no *Ac*. An exceptional kernel ap-

peared on the ear produced by this cross. It was *I sh* in phenotype. The plant grown from this kernel exhibited the recessive bronze phenotype, and no mutations to *Bz* were noted in the plant tissues. One ear of this plant was self-pollinated, and from the kernel types that appeared it could be concluded that one chromosome 9 carried *I*, *sh*, and *bz* and its homologue carried *C*, *sh*, and *bz*. Another ear of this plant received pollen from a plant that was homozygous for *C*, *sh*, and *bz* but also carried one *Ac*. On the ear produced by this cross it was evident that *Ds* was present in the *I sh bz*-carrying chromosome, and also that mutations to *Bz* were occurring at the *bz* locus in this chromosome, but only in those kernels that had received *Ac* from the male parent. Subsequent tests indicated that the female parent did not have *Ac*, but that the *bz* locus carried in the *I sh bz* chromosome was capable of mutating to higher alleles of *Bz* in its presence. Tests were then conducted to determine the nature of the change in the *Sh Bz* region that had originally occurred in one of the *I Ds Sh Bz*-carrying chromosomes of the parent plant, and also the conditions that governed the reversions to *Bz*. The evidence obtained from these tests is summarized below.

A *Ds*-induced mutation in an *I Ds Sh Bz*-carrying chromosome affected gene action in the segment of the chromosome that includes *Sh* and *Bz*. *Ds* was not altered in location by this event, and thus the segment was composed of the recognizable components *Ds*, *sh*, and *bz*. In all subsequent tests, this segment behaved as a unit in inheritance, for no evidence was obtained of crossing over within it. Chromosomes carrying it were normally transmitted through both pollen and egg, and individuals homozygous for it were viable and normal in appearance. In the absence of *Ac*, no modifications affecting this segment occurred; there were no dicentric-acentric-chromatid-forming events at *Ds*, and no mutations to *Bz*. In its presence, however, both occurred. The frequency

of occurrence of dicentric chromatid formation was high, and that of mutation to *Bz* was low, but it was evident that both events were expressions of the presence of *Ds* in this segment and of its responses to *Ac*. The *sh* component of the segment was stable both in the presence and in the absence of *Ac*, for no mutations to *Sh* were noted. In the presence of *Ac*, a few mutations to *Bz* occurred in sporogenous cells. These were detected in individual kernels appearing on ears produced by appropriate crosses of plants that had the modified segment. Thirteen kernels representing independent occurrences of germinal mutation to *Bz* were selected from such ears, and examination of the mutant commenced with the plants derived from them. In none of the thirteen cases did the event responsible for the mutation to *Bz* affect the recessive *sh* expression, nor did it result in a change in location of *Ds*. In the absence of *Ac*, the *Bz* expression proved to be stable; but in its presence further mutations occurred, many of them again giving rise to the recessive, *bz*, expression. Some of these *bz* revertants, in turn, were unstable, and there were many mutations back to *Bz*, but only when *Ac* was present in the nucleus.

Chromosomes carrying any one of the thirteen examined *Bz* mutations were normally transmitted through pollen and egg, and plants homozygous for them were viable. The mutants, however, were not all alike. The intensity of pigmentation in some of them was less than that produced by the standard *Bz*. Also, most of the mutants did not produce the diffusible substance that allows a *Bz* phenotype to appear in a *bz* genotype, as the standard *Bz* is known to do. This substance is made evident in kernels that are sectorial for the *Bz* and *bz* genotypes. Cells in a *bz* sector that are immediately adjacent to a *Bz* sector exhibit a *Bz* phenotype because of the presence in them of a substance derived from the *Bz* cells. A few of the thirteen *Bz* mutants did produce this substance, and in all major respects



these mutants were similar to the standard *Bz*. Two of the thirteen mutants received extensive study. In both, the *Ds sh Bz* segment behaved as a unit in inheritance. It could be shown, however, that the *Ds* component was located at a position distal to that of the *Bz* component, even though the two components were not separated from each other by crossing over in rather extensive tests designed to detect such separation.

This evidence accords with that obtained in all other examined cases of change in gene expression produced by *Ds* after its insertion at this one particular position in chromosome 9—just distal to *Sh*. In the presence of *Ac*, it can induce various types of change in gene action. Some of them effect a spread of mutation-type changes along the chromosome to either side of *Ds*. The extent of influence of any one event varies from an effect on *Sh* alone to an effect on all the genic substance located within the *I*-to-*Bz* interval, including these two loci. In all the many examined instances of such change in gene expression, the *Ds* element has been found to be present after the occurrence of the mutation-producing event, and its location has apparently been unaltered. In all cases of reversion in the gene expression, it has been shown that the *Ds* element was responsible and, again, that its position was not altered.

#### CONTINUED EXAMINATION OF THE *a<sub>1</sub><sup>m-1</sup>Spm* SYSTEM OF CONTROL OF GENE ACTION

Since the mode of control exhibited by the *a<sub>1</sub><sup>m-1</sup>Spm* system was outlined in some detail in Year Book No. 54 it need not be reviewed here. During the past year, studies of this system were aimed at adding to the evidence about transposition of the *Spm* element by determining the extent to which it occurs at various stages in plant development. It was learned that, although transpositions of *Spm* may occur early in plant development, most of

them occur relatively late. Progeny of plants carrying an *Spm* element at a known location in one chromosome of the complement were investigated, in order to determine the frequency of occurrence of transposition from this known location—in either chromosome 5, chromosome 6, or chromosome 9—to new locations. Disappearance of *Spm* from the known location and its appearance at a new location were detected in some individuals in each progeny.

Several different modifiers of the *a<sub>1</sub><sup>m-1</sup>Spm* system were also examined. One behaves as a recessive in the presence of *Spm*; in the absence of *Spm* it controls the type of *a<sub>1</sub><sup>m-1</sup>* action in plant and kernel. The plant tissues develop pigment much as they do in the absence of *Spm*, as described earlier, although the rate of development is much slower. The kernels, however, are usually totally colorless; in some of them, one or several very small dots of deep pigmentation may appear. Several modifiers of this type have been observed in the *a<sub>1</sub><sup>m-1</sup>* cultures, and each was found to occupy a different position in the chromosome complement. Another type of modifier that has appeared greatly enhances the frequency of occurrence of mutation at *a<sub>1</sub><sup>m-1</sup>*, but only when *Spm* is also present in the complement. A third type of modifier is a system composed of two complementary elements, which are independently located in the chromosome complement. Its effects are observed in the absence of *Spm*. One of the two elements of this system is responsible for the appearance in the kernel of a regular pattern of presence and absence of a pale-colored anthocyanin pigment. When the second factor is also present, dots of deep anthocyanin pigmentation appear in the colorless areas.

It is clear that a number of different elements may be present in the nucleus, each involved in some manner in control of *a<sub>1</sub><sup>m-1</sup>* action. In studies of the *Ds-Ac* system, on the other hand, such distinctive types of modifiers have not yet been rec-



ognized. Analysis of that system has therefore been relatively free from the apparent confusion that such modifiers can introduce in attempts to understand the modes of action of particular controlling systems. Nevertheless, recognition of the presence of a number of different types of element, each of which can act upon a particular known controlling element at a given gene locus, is of considerable significance in viewing the modes of operation of controlling elements and their integrative action in the nucleus. Just such complexity of relations is to be expected if controlling elements play a significant part in modifying gene action within the nucleus. If such modifiers were not found, each system would seem to behave as an isolated unit, and its relation to integrative mechanisms within the nucleus would not be apparent. Various levels of integration of controlling systems are to be expected. The modifiers described here may represent a second level of integration.

#### CHANGES IN CHROMOSOME ORGANIZATION AND GENE EXPRESSION PRODUCED BY A STRUCTURALLY MODIFIED CHROMO- SOME 9

A structural modification affecting the organization of chromosome 9, which is responsible for inducing other structural alterations both in chromosome 9 and in other chromosomes of the complement, has been examined. In this case, the substance of a normal chromosome 9 is divided into two chromosomes of quite different lengths. The smaller chromosome is composed of the distal third of the short arm of the normal chromosome 9, and carries the loci of *Yg* and *C*. At its proximal end is a centromere, from which extends a short piece of deeply staining chromatin of unknown origin; but the extension is often lost from the chromosome, leaving it with a terminal centromere. This short member of the structural modification will be referred to as the fragment chromosome. The longer member is composed of

the proximal two-thirds of the short arm of chromosome 9 and all of its long arm. The locus of *Sh* is close to the end of the short arm of this chromosome, and the loci of *Bz* and *Wx* follow in the normal order. This member will be referred to as the deficient chromosome.

Cytological examination of various meiotic stages was made in plants that were either heterozygous or homozygous for this structural modification. In the heterozygote, the deficient chromosome was always synapsed with homologous parts of the normal chromosome 9 throughout all of its long arm, and usually also throughout most of its short arm. At the pachytene stage, the fragment chromosome in most cells was found to be synapsed with homologous parts of the normal chromosome 9 throughout much of its length. In some cells, however, it was completely unassociated, lying free in the nucleus. Particular attention was given to those cells in which synapsis of both components of the modified chromosome 9 with homologous parts of the normal chromosome was fully expressed. They furnished no evidence of either duplication or deficiency of parts of chromosome 9 within either of the two components of the structurally modified chromosome; the fragment chromosome and the deficient chromosome appear to represent a complete chromosome 9. It should be emphasized, however, that, in many of the cells in which the fragment chromosome was completely synapsed with its homologous part in the normal chromosome, the centromere of the fragment was closely appressed to the adjacent region in the normal chromosome, where *Sh* and *Bz* are located. When this association was observed, the *Sh*- and *Bz*-carrying region in the deficient chromosome, located near the end of its short arm, was not synapsed with the homologous region in the normal chromosome.

The deficient chromosome is transmitted through the pollen grain only when the fragment chromosome is also present in the tube nucleus. Through the female

gametophyte, however, it is transmitted without the accompanying fragment. Thus, plants may be obtained that have a normal chromosome 9 and a deficient chromosome 9 but no fragment. When the deficient chromosome carries *Sh*, *Bz*, and *Wx* and the normal chromosome carries the recessive alleles, crossing over within the *Sh*-to-*Bz* and *Bz*-to-*Wx* regions may be determined readily by using plants of this constitution as pollen parents in crosses to plants that are homozygous for *sh*, *bz*, and *wx*. Among a total of 16,514 kernels obtained from crosses of this type, only 0.4 per cent carried a chromosome that could have undergone crossing over within the *Sh*-to-*Bz* region. This figure represents a marked reduction from the standard value of 1.5 to 2 per cent. Within the *Bz*-to-*Wx* region, however, the standard frequency of crossing over was exhibited, amounting to 18.6 per cent. All but 5 of the kernels from these crosses were *sh* in phenotype. The 5 exceptional kernels were all *Sh Bz Wx* in phenotype.

A number of crosses similar to those just described were conducted with plants that had a fragment chromosome in addition. In these plants, the deficient chromosome carried *Sh* and *Wx* and the normal chromosome carried the recessive alleles, *sh* and *wx*. They were crossed to plants that were homozygous for *sh* and *wx*. Because of nonregulated disjunctions of the fragment chromosome at the first meiotic anaphase, and also because of frequent noninclusion of the fragment in either a telophase I or a telophase II nucleus, the number of pollen grains that carried both the deficient chromosome and the fragment was considerably lower than the number that carried a normal chromosome, with or without the fragment. Because the deficient chromosome carrying *Sh* is transmitted through the pollen only when the fragment is also present, the number of *Sh* kernels on ears produced by a test cross of this kind should be considerably smaller than the number of *sh* kernels. Also, because a crossover in the distal third of the

short arm—between the normal chromosome and the fragment—interferes with a crossover in the region proximal to it, and because such a crossover leads to chiasmal association of the fragment and the normal chromosome at metaphase I and consequently to disjunction of the fragment at anaphase I and its inclusion in two of the four nuclei at telophase II, the percentage of pollen grains carrying the fragment and the deficient chromosome with a crossover in the *Sh*-to-*Wx* region (the *Sh wx* class) should be smaller than the percentage of pollen grains carrying a normal chromosome with the reciprocal crossover (the *sh Wx* class). In the light of these statements, the ratios of kernel types appearing on the ears produced by the test cross may be readily interpreted. They were as follows: 4072 *Sh Wx*:524 *Sh wx*:4319 *sh Wx*:20,081 *sh wx*.

A test of the above-described types was conducted with five plants that had the markers *c*, *sh*, *Bz*, and *wx* in the normal chromosome 9, *c* in the fragment chromosome, and *Sh*, *Bz*, and *Wx* in the deficient chromosome. When they were used as pollen parents in crosses with plants homozygous for *C*, *sh*, *bz*, and *wx*, an unexpected class of kernels appeared. These kernels exhibited the recessive *bz* phenotype, and all of them were *sh*. They appeared in constant proportions, as shown in section B of table 4. Another plant, of similar constitution with respect to markers in the normal chromosome 9 and the deficient chromosome but having no fragment present, was also crossed to plants homozygous for *C*, *sh*, *bz*, and *wx*; and from this cross no *bz* kernels resulted, as shown in section A of the table. Apparently, the fragment is in some way responsible for the appearance of kernels exhibiting the *bz* phenotype. This was also suggested by a test conducted with plants that had *c*, *sh*, *Bz*, and *wx* in the normal chromosome 9, *C* in the fragment, and *Sh*, *Bz*, and *Wx* in the deficient chromosome. They were used as female parents in crosses with plants that were homo-

zygous for *c*, *sh*, *bz*, and *wx*. Among the 506 kernels on the ears produced, 377 were *c* (53 *Sh Wx*:12 *Sh wx*:18 *sh Wx*:294 *sh wx*) and 129 were *C*. Among the *C* kernels, the expected phenotypes appeared (84 *Sh Bz Wx*:4 *Sh Bz wx*:1 *sh Bz Wx*:35 *sh Bz wx*), but in addition there were 5 exceptional kernels, *C sh bz wx* in phenotype. The appearance of kernels exhibiting the *bz* phenotype might be explained on the assumption that the fragment chro-

was responsible for the altered *Bz* expression, the 41 *bz* kernels entered in table 4, as well as 4 of the 5 *bz* kernels produced by the second cross, were sown and plants were obtained from them. All forty-five plants exhibited the *bz* phenotype. Sporocytes were collected from all the plants, and from them the chromosome 9 constitution in each plant could be determined. Forty-four of the forty-five plants had two normal-appearing chromosomes 9; in

TABLE 4

DATA SHOWING THE FREQUENCY OF APPEARANCE OF THE *bz* PHENOTYPE IN PROGENY OF PLANTS CARRYING THE FRAGMENT CHROMOSOME (*B*, BELOW), AND THE ABSENCE OF THIS PHENOTYPE IN PROGENY OF PLANTS THAT DID NOT HAVE THE FRAGMENT CHROMOSOME (*A*, BELOW)

- A. ♀ *C sh bz wx*/*C sh bz wx* × ♂  $\frac{sh\ Bz\ wx; \text{normal chromosome 9}}{Sh\ Bz\ Wx; \text{deficient chromosome 9}}$   
 B. ♀ *C sh bz wx*/*C sh bz wx* × ♂ Same as A, but fragment also present

PHENOTYPE OF KERNEL	A	B (PLANTS 1 TO 5)					Totals for B
		1	2	3	4	5	
<i>Sh Bz Wx</i> .....	0	81	141	147	169	249	787
<i>Sh Bz wx</i> .....	0	4	15	12	27	33	91
<i>sh Bz Wx</i> .....	71	65	81	95	147	154	542 *
<i>sh Bz wx</i> .....	367	357	335	454	480	602	2229
<i>sh bz Wx</i> .....	0	2	1	1	3	2	9 †
<i>sh bz wx</i> .....	0	5	3	6	6	12	32
Totals .....	438	514	576	716	832	1052	3690
% <i>bz</i> among <i>sh</i> class.....	0	1.6	0.95	1.2	1.4	1.8	1.4

\* Equals 19.5 per cent of *sh Bz* class.

† Equals 21.9 per cent of *sh bz* class.

mosome carries *bz* and that crossing over occurs between this marker and the centromere of the fragment. This explanation would require that the fragment carry a duplicated piece of chromatin, for it is known that the loci of *Sh* and *Bz* are in the deficient chromosome. But, as was stated earlier, no evidence of such a duplication was seen. Moreover, if this explanation were correct, a crossover in this region would be expected to interfere with one to the right of it—between *Bz* and *Wx*. No such interference was expressed, as the data in table 4 indicate.

In order to initiate investigation of the nature of the change in chromosome 9 that

seven of the forty-four, the fragment chromosome was also present. No modification in the *bz*-carrying region of one chromosome 9 was obvious in any of these plants. In the remaining plant of the forty-five, however, a modification was clearly observed. This plant had one normal chromosome 9, a fragment chromosome, and a deficient chromosome; but a small piece of chromatin was missing from the end of the short arm of the deficient chromosome. The loci of *Sh* and *Bz* had been deleted, and because of this the *sh bz* phenotype had appeared. It is clear, nevertheless, that most changes from *Bz* to *bz* are not associated with any gross change in chromo-



some composition. It is possible that during the meiotic process, when the fragment is synapsed with the normal chromosome, the fragment becomes joined to the normal chromosome, at the region of its centromere, by a mechanism that simulates the crossover process. This region of joining would be situated at the part of the normal chromosome containing the loci of *Sh* and *Bz*. It is known that the fragment may attach itself at its centromere region to ends of chromosomes, with associated loss of its centromere activity. In the homozygote, the fragment can thus join with the end of the deficient chromosome, and so re-establish a structurally normal chromosome 9. Twelve independent cases of this event have been examined. They were detected because of change in expression of *Sh*, or of both *Sh* and *Bz*, in the deficient chromosome to produce either *sh* or the double mutant *sh bz*. In these twelve cases there was no evidence of deficiency in the *sh*- or the *sh bz*-carrying region of the reconstructed chromosome 9, or of any centromere activity at the position of union. The reconstructed chromosome behaved in mitosis like a normal chromosome 9. Even though the evidence obtained so far is not extensive, it is sufficient to indicate that the fragment chromosome is responsible for initiating the described modifications in gene expression in the *Sh*-and-*Bz*-carrying region of chromosome 9.

The fragment chromosome initiates types of modification other than those described above. The events responsible for them may occur in either somatic or sporogenous cells. Cytological examinations were made of sixty-two plants derived from kernels whose phenotypes had suggested alteration in constitution of the fragment chromosome itself. From these observations it was possible not only to learn what types of change in constitution of the fragment may occur but also to discover some of the alterations the fragment can induce in the constitution of other chromosomes of the complement. The modifications observed include nondisjunction of the fragment; "misdivision" of its centromere, resulting in isochromosome formation; ring-chromosome formation; attachment of the centromere of the fragment to the centromere of another chromosome of the complement, effecting union of the fragment with one arm of the other chromosome; and, as mentioned above, attachment of the centromere of the fragment to the end of another chromosome, effecting union of the fragment with this chromosome. Other, more complex types of interchromosomal modifications involving the fragment were also noted. The times and frequencies of occurrence of these events appear to be under genetic control. That tissues of plants carrying the fragment often exhibit a rather precise pattern of occurrence of such events indicates a regulated frequency.

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## DEPARTMENT OF ARCHAEOLOGY

*Cambridge, Massachusetts*

H. E. D. POLLOCK, *Director*

In last year's annual report (Year Book No. 54) we noted the completion of a program of field activities concerned with the peninsula of Yucatan. The past year has seen the staff engaged in the preparation of preliminary reports covering the work of the 1955 field season and the beginning of the larger, definitive studies that will follow.

The handling of archaeological collections and recorded data is, naturally, determined by the nature of the materials. Buildings cannot be collected, nor can excavations be left open indefinitely without undue risk of deterioration of the exposed remains. Architecture must, then, be recorded in such detail that all pertinent data will be available after the worker has left the site of his excavations. The same is true, also, of large sculptures and monuments. Pottery is transportable, but it frequently occurs in such amounts that only preliminary sorting and classification can be done on the spot. Final study, when the full range of material is at hand, must await a later time. Other artifacts, covering a large range of relatively imperishable materials, also may occur in considerable numbers. In the busy press of an active field season these objects can receive only preliminary cataloguing, and they too, like the pottery, must be set aside for later, more painstaking study.

The problems of handling collections and data indicated in the preceding paragraph have been met in a variety of ways. Those members of the staff concerned with architecture, and with the wider implications of building remains, such as population estimates, settlement patterns, and the life and customs of the people in general, have been at work in Cambridge. Over the years of active field work our collections of pottery and other artifacts have steadily moved from the site of excavation

to a central repository in Merida, where they were systematically stored. To study these collections, certain members of the staff traveled to Yucatan this past winter. Human skeletal remains, many extremely fragile, were stored at our field camp, and the study of this material by a physical anthropologist also required his going to Yucatan. Lastly, we should mention collections that could be shipped to the United States for study by scholars in fields other than archaeology. These materials included animal bones, selected specimens of shell and stone, fragments of textiles, and all artifacts of metal.

In July 1955, Edward I. Fry, a graduate student in anthropology at Harvard University, spent several weeks at the Department's field camp in Telchaquillo, Yucatan, studying our collections of human skeletal remains. Because of the nature of the material he concentrated his attention on evidences of pathology, anomalies, and deformation exhibited by the remains rather than attempting to accumulate extensive amounts of anthropometric data. Fry's report on his findings is now in press. Highlights include comparative data on types of tooth filing, the low incidence of carious teeth and of healed fractures of long bones, and the high incidence of anteroposterior cranial flattening and of degenerative arthritis.

R. E. Smith and Proskouriakoff went to Merida in December 1955, the former to work with our pottery collections, the latter to study our other collections of artifacts. Proskouriakoff returned to Cambridge early in March, and is now engaged in preparing a definitive report on her work. Smith, faced with a very large amount of material, remained in Yucatan until the end of May. Statements covering the work of Smith and Proskouriakoff will be found below.

A minor exception to the ending of field activities in 1955 was the collecting of pottery samples at the ruins of Uxmal and Kabah during the past winter. These small excavations, which were carried out by Strömsvik during February, were designed to provide R. E. Smith with background material for his studies of Mayapan ceramics. The results of the work are reported by him. As our five-year contract with the Mexican Government had expired in September 1955, Strömsvik's excavations were made under special permission from the Instituto Nacional de Antropología e Historia, and in co-operation with its work at those sites.

It has been mentioned above that studies of parts of our collections have been undertaken by scholars in fields other than archaeology. These studies are listed by Proskouriakoff in a subsequent section. Particularly worthy of note are the spectroscopic analyses of metal objects, some of which will be subjected to further tests, by Dr. W. C. Root, of Bowdoin College, and the identification of large collections of animal bones by Mr. Clayton Ray, a graduate student at the Museum of Comparative Zoölogy of Harvard University. Although the work on the metals has not progressed sufficiently for any details of the findings to be given, this study holds promise of indicating routes and sources of trade and commerce; no metal being native to the Yucatan peninsula, all objects of this material must have been imported.

In the matter of the identification of animal bones produced by our excavations, the Department was most fortunate in obtaining the assistance of Ray, who has made over 6000 identifications of mammals, reptiles, amphibia, fish, and birds. It is pleasant to relate that, aside from the anthropological information to be derived from these identifications, such as the dietary habits of the ancient Maya and the domestication of animals by these people—studies yet to be made—our collections were of sufficient interest to another discipline to merit the preparation by Ray of

two papers to be published in zoological journals.

While referring to assistance from other disciplines, we wish to report the dating by the carbon-14 method of two charcoal samples from Mayapan. The age determinations were made in 1955 by Professor Hessel de Vries, of the Physical Laboratory, State University of Groningen, Holland. The specimens were selected in the hope of marking, as nearly as possible, the beginning and ending of the principal period of occupation of the city. The precise provenience and other details relative to the samples will be given in a report now under preparation. Suffice it to say here that the earlier specimen (GRO 452) yielded a reading of 700 (A.D. 1255)  $\pm 95$  years. This finding is in close accord with the date expected on archaeological grounds. The later specimen (GRO 450) yielded a reading of 335 (A.D. 1620)  $\pm 90$  years, approximately 200 years later than the expected date and falling in post-Columbian times.

During the past year Ruppert and A. L. Smith have been occupied primarily with the preparation of preliminary reports covering their extensive excavations in dwelling remains in Mayapan and their reconnaissance and excavation of similar remains elsewhere in Yucatan. One such report is now in press. They have also begun the analysis of the large amounts of data accumulated during five seasons of field operations at Mayapan and other ruins, an analysis that should materially increase our knowledge of the character of domestic architecture, patterns of settlement, and sizes of populations in Mayapan and vicinity.

Thompson, in addition to preparing a report on his excavations at Mayapan in 1955, has continued work on his catalogue of Maya hieroglyphs (see Year Book No. 50, pp. 244-245). The end of this long and arduous undertaking is now in sight.

During the latter half of the period under review Shook was on leave of absence from the Institution. This leave was to

enable him to lead an expedition of the University Museum, University of Pennsylvania, to the great lowland Maya ruins of Tikal in Guatemala. Before his departure he was engaged in preparing reports covering his activities in Yucatan during the 1955 field season.

Sanders, whose fellowship with the Institution ended January 1, 1956, has been occupied with the preparation of a report on his archaeological survey of the east coast of Yucatan, a survey that extended over two field seasons. His report is expected in the near future.

Shepard has been pursuing studies in geology and mineralogy to broaden her background in those subjects. This work has included a research project on clay mineralogy in which she is applying X-ray and petrographic methods to the study of the thermal behavior of clays within the temperature range of primitive firing. Her survey of technical methods in archaeology has been supplemented by abstracting for *Studies in Conservation*, International Institute for the Conservation of Museum Objects; she is serving on R. J. Gettens' American working team. Shepard has also prepared sets of models demonstrating the factors affecting the color of fired clay for the purpose of clarifying basic principles of ceramics for archaeological students.

The foregoing covers the research activities of the staff. There are some administrative matters which should be reported. Most important is the decision of the Institution to discontinue its archaeological activities some two years hence.

Our five-year contract with the Mexican Government, under which our program of field activities in that country had been carried out, expired in September 1955. As excavations were at an end, and the terms of the contract allowed a period of two years following the completion of the work before our collections need be turned over to the Instituto Nacional de Antropología e Historia, renewal of the contract was not requested.

By late winter 1956 it was apparent that

our field camp at Telchaquillo was no longer needed. During March and April all equipment at the camp was moved to Merida, and the property, which we held on lease, was returned to the owner. This operation was carried out by Strömsvik.

Before the closing of the Telchaquillo camp, it had been decided by the Institution that the greater part of the field equipment no longer needed by the Department should be turned over to the Instituto Nacional de Antropología e Historia, to be used in its archaeological researches in the Maya region. This policy, which assured that the equipment would continue in the uses for which it originally was purchased, has resulted in the handing over of considerable amounts of highly useful equipment to the Yucatan office of the Instituto Nacional.

Toward the end of April the President of the Institution and the Director of the Department made a journey of approximately a week's duration to Yucatan, Guatemala, and Honduras in order to review some of the past activities of the Institution in the field of Middle American archaeology. The ruins of Uxmal, Kabah, Chichen Itza, and Mayapan were visited in Yucatan, where the President and the Director were joined by R. E. Smith and Strömsvik. In Guatemala the great ruins of Tikal, the largest known Maya city, were inspected with the aid of Shook, who was just completing his field season there under the auspices of the University Museum of the University of Pennsylvania. The following day, again in the company of Shook, the ruins of Copan in Honduras were visited. Lastly, the fine Museo Nacional de Arqueología y Etnología de Guatemala, in the organization of collections and the installation of exhibits for which the Institution had played a prominent role, was inspected.

The fifty-fourth annual meeting of the American Anthropological Association, held in Boston during November 1955, included a symposium on Middle American anthropology and thus was of particu-



lar interest to our staff. Papers were presented by Proskouriakoff and Thompson; Shook acted as discussant of a paper; other papers were given by a number of people who at one time or another have been associated with the Department.

During the past year Proskouriakoff has been appointed Assistant Editor (Middle America) of *American Antiquity*, replacing Thompson.

It is with deepest regret that we report the death on June 24, 1956, of Earl Halstead Morris, a recently retired member of the staff of the Department. His outstanding accomplishments in the fields of Southwestern and Middle American archaeology were described (Year Book No. 54) at the time of his retirement. American archaeology has suffered a grave loss with his death.

We also have the sad task of reporting the death on March 22, 1956, of George Alfred Leon Sarton, head of the Section of the History of Science under the Division of Historical Research. Sarton's long and brilliant career is reviewed in our annual report for 1948-1949 (Year Book No. 48). He will be sorely missed by his colleagues and by all who knew him.

In recognition of extensive research work and brilliant publication, J. Eric S. Thompson was awarded the Viking Medal in archaeology for 1955. Thompson is the second member of the staff of the Department to receive this award since its inception ten years ago. The first recipient of the medal was Alfred Vincent Kidder, then Chairman of the Division of Historical Research.

It is a pleasure to be able to record yet another honor for our past Chairman. In October 1955, Dr. Kidder received from the University of San Carlos, Guatemala, the degree of *Doctorado honoris causa*.

#### CERAMIC STUDIES IN YUCATAN

R. E. SMITH

During the past field season Smith's work encompassed three main ceramic projects:

a study of the surface material from Mayapan; the analysis of pottery from a number of test trenches dug by Strömsvik at Uxmal and Kabah; and a review of three wares (X Fine Orange, Tohil Plumbate, and Peten-like polychrome) well represented in the Alberto Márquez collection in Merida. In addition, ceramic summaries of the pottery from Dzab-Na and from Xcaret were made, respectively, for Strömsvik and for E. Wyllys Andrews.

The principal wares and forms found on the surface at Mayapan are representative of the latest ceramic phase at the site. They may be divided into six main wares: unslipped porous gray or cinnamon, slipped Mayapan Red, Mayapan Red-and-buff, Mayapan Red-on-buff or -orange, Mayapan Red-and-black-on-buff or -orange, and V Fine Orange. The unslipped forms include low-necked, wide-mouthed jars with lightly striated bodies; huge high-necked, bolster-rim jars, usually thickly coated with plaster; censers of three types, namely, effigy, ladle, and jar shapes, either tripod or with pedestal bases; effigy vessels in the form of turtles, humans or gods, and frogs; figurines; and some bowls and dishes. The effigy and jar censers, effigy vessels, and figurines are usually painted, and most have appliquéd adornos. The slipped forms include jars, both water and storage; tripod dishes and bowls; flat-based bowls, both open-mouthed and restricted; basins with bolster rims and horizontal strap handles; and in the case of V Fine Orange (Berlin, 1956, Carnegie Inst. Wash. Pub. 606, Contrib. 59), tripod basal-flanged bowls or dishes. The use of feet in the latest Mayapan phase (as well as in all earlier Mayapan phases) is prolific. Such supports, three, never four, in number, are associated with nearly all forms, even, though rarely, with jars. The pottery, both slipped and unslipped, is closely comparable to the latest pre-conquest material found on the east coast of the Yucatan peninsula.

In the eight trenches dug at Uxmal, the pottery recovered amounted to about 25,000

sherds. All were reviewed rapidly, and about 14,000 were actually studied, material from the three best-stratified trenches, comprising fourteen cuts in all, being used. All the pottery examined appears to be late in the Puuc sequence. There are six main wares: unslipped light brown, Medium Slate, Thin Slate, Puuc Red, Holactun Slate, and Z Fine Orange. The principal forms associated with the unslipped ware are coarsely striated jars and spiked censers with flange and pedestal bases. Medium Slate forms are water jars, bolster-rim basins with vertical strap handles, flaring- or outcurving-sided dishes, and incurved-rim ring-stand bowls. Thin Slate forms, which differ somewhat from Medium Slate, are round-sided bowls with direct or bead rim (often with three nubbin feet), cylinder vases with flat or pedestal bases, restricted-orifice bowls, flaring- or outcurving-sided tripod dishes, deep bowls, and basins. Puuc Red shapes, much the same as those of Thin Slate, are round-sided bowls with direct or bead rim (rarely with pedestal or three hollow oven feet), flaring- or outcurving-sided tripod dishes, cylindrical vases, and flaring-sided bowls rounding to flat base (mostly with moldmade carving). Holactun black-on-cream ware has very distinct forms, including bolster- or thickened-rim jars, T-bolster-rim basins, and incurved- and bolster-rim bowls.

The ceramic picture at Kabah, as shown by our findings, differs from that at Uxmal in that there is evidence of a sequence comprising an early and a late phase, and possibly a middle phase. Z Fine Orange and Holactun black-on-cream are found in the upper and middle levels but not at the bottom, where a small quantity of Peten-like Early Classic polychrome types occurs. Many other differences between the Kabah and Uxmal material will be presented in a later publication.

The recording of the X Fine Orange, Plumbate, and Peten-like polychrome wares from the Márquez collection is important, even though precise data as to the circumstances under which the pieces

were discovered are lacking. As the specimens are recognized ceramic types, they add to the body of information regarding those types. This is particularly true of categories of pottery that are known only in the form of sherds, whole pieces such as these shedding light on vessel shapes and systems of decoration. Finally, it is always well to make record of material in private hands, for not only is this often difficult of access to students, but also there is danger that it may become scattered and lost to science.

A few words will suffice for the material from Dzab-Na and Xcaret, since the work done at these sites is being published by the respective excavators. Dzab-Na is a cave and cenote located near the town of Tecoh. The pottery collected in the cave was not stratigraphically separated but was removed as a unit. It consists of pre-Classic, Puuc and Peten-like Classic, and Mayapan Period pottery, in that order of importance. Xcaret is a large site on the east coast of the Yucatan peninsula, opposite the northern extremity of Cozumel Island. Ceramically, Xcaret appears to be primarily of Puuc type: although Mayapan Period and even Recent types, as well as Peten-like polychromes and a few pre-Classic specimens, are present, the great majority of sherds belong to the Puuc tradition.

#### ARTIFACTS OF MAYAPAN

TATIANA PROSKOURIAKOFF

Archaeological work in recent years has been centered largely on architecture, settlement patterns, and pottery collections. Stone tools and other small artifacts that would throw light on the technologies of the peoples concerned have received very little study. Only major excavation projects yield collections of a size that can provide an adequate sample of tools used by a given people. Comparison and even description of the technologies of various Middle American cultures still await necessary reports of relevant data. Such reports are available for only a very few

sites in the Maya area and are completely lacking for most of Mexico. A. V. Kidder's studies of the artifacts of Uaxactun and of Kaminaljuyu, and Woodbury's description of those from Zaculeu, give us information on the tools used by the Maya during the Classic period in the Peten and in the highlands. The collection from Mayapan will extend our knowledge to the post-Classic period in Yucatan.

Unfortunately, literature is particularly deficient in data that would acquaint us with locally antecedent techniques, but the Institution's unpublished catalogue of objects from Chichen Itza, and scattered pieces in museum collections, provide some background for judging the effect of the violent events of the post-Classic era on the material culture and resources of the people of Mayapan. The present study, however, remains primarily descriptive, and is concerned not so much with the history of particular techniques or the tracing of trade relations as with the total complex of tools and ornaments characterizing a particular stage of Maya culture. For this purpose, technical identification of the material of every specimen, although highly desirable, was not deemed essential, and expert advice was sought only on selected samples. Metal was the only exception; the entire collection of metallic specimens was brought to the United States for analysis, and Dr. William C. Root, of Bowdoin College, is now examining it both for composition and for working technique. Mr. Robert Berman has given us help with the identification of samples of stone imported to Mayapan from other regions, and Dr. W. J. Clench, of Harvard University, has examined and identified varieties of shell. In addition, Miss Joy Mahler has helped us with descriptions of fragments of textiles, and Mr. Clayton Ray with animal bones used for artifacts.

Although the study has barely passed its initial stages, a general view brings out interesting contrasts with other known collections. For example, highly specialized forms of large stone tools found at Maya-

pan are comparable to those at other sites, but the number of roughly shaped tools used in stone working and plaster smoothing far exceeds what has been reported elsewhere.

The most radical changes are reflected in the flint-working trade. Techniques seem to decline, and the materials used are inferior. The absence of the large chipped celt with a polished edge which is found in earlier Yucatan collections may point to a significant change in cultivation procedure. A common small celt form, on the other hand, appears as a new feature. Small arrowheads probably mark the introduction of the bow in warfare or hunting, and the rarity of larger stemmed points may mean a decline in the use of the spear thrower.

Obsidian flake blades were manufactured in great numbers, but there are few chipped implements. The fact that green obsidian, common in late periods in Mexico, is even more rare here than in early times in the Guatemala highlands suggests that, contrary to expectation and in spite of historical evidence of Mexican settlements at Xicalango, Mexican traders were not conspicuously successful in the Maya capital.

The collection of jades and other fine stone ornaments from Mayapan is disappointingly meager; this scarcity is due mainly, no doubt, to the fact that so many caches and tombs had been looted, but even from our small collections it is clear that few such objects were manufactured locally and that the best pieces were antiques when they were acquired.

Shell, bone, tooth, antler, and other organic materials exhibit ancient traditional techniques, which our shell collection illustrates somewhat better than other groups, containing, as it does, a large number of rejected pieces that often show technical process more clearly than does the finished product.

Proskouriakoff spent two months in Merida this season classifying and studying the collections. Over 250 photographs



of specimens were made by Sr. Raul Acuña, of Merida, to serve as illustrations for the report now in preparation, which is designed to make this material available for comparison with collections from other cultures.

#### PUBLICATIONS

H. E. D. POLLOCK

Two books, mentioned in our report for last year as in progress, have now appeared. *Archaeological Reconnaissance in Central Guatemala* (Publication 608), by A. Ledyard Smith, was published in November 1955. The book was composed on IBM typewriter and printed by offset throughout. It covers sixty-eight sites in the Guatemala highland area; some were explored briefly, others were mapped and excavated in varying degree of intensity.

*Ceramics for the Archaeologist* (Publication 609), by Anna O. Shepard, appeared in June 1956. It was composed on the Photon photographic typesetting machine and printed by offset. It discusses the composition, sources, and properties of ceramic materials, ceramic processes and techniques of prewheel potters, ceramic analysis and description, problems of pottery classification, and interpretation of ceramic data. Five appendixes include more technical details. A list of titles for suggested reading is appended to the bibliography.

The third paper in volume XII of *Contributions to American Anthropology and History* (Publication 606), *Late Pottery Horizons of Tabasco, Mexico*, by Heinrich Berlin, appeared in the summer of 1956.

A manuscript by Ralph L. Roys, *The Political Geography of the Yucatan Maya*, was submitted for publication in March 1956.

Five papers have been added to the fifth volume of *Notes on Middle American Archaeology and Ethnology: Two New Gallery-Patio Type Structures at Chichen Itza* (no. 122), by Karl Ruppert and A. Ledyard Smith; *Easter Ceremonies at Santiago Atitlan in 1930* (no. 123), by Elsie McDougall; *Pottery Specimens from Guatemala: II* (no. 124), by Robert E. Smith; *Pottery Vessels from Campeche* (no. 125), by Robert E. Smith; *Selected Pottery from Tabasco* (no. 126), by Heinrich Berlin.

The second volume of *Current Reports* has continued with the addition of eight papers: *A Noble's Residence and Its Dependencies at Mayapan* (no. 25), by Donald E. Thompson and J. Eric S. Thompson; *Early Ceramic Horizons at Mayapan and Santa Cruz* (no. 26), by Robert E. Smith; *Another Round Temple at Mayapan* (no. 27), by Edwin M. Shook; *An Altar and Platform at Mayapan* (no. 28), by Donald E. Thompson; *A Residential Quadrangle—Structures R-85 to R-90* (no. 29), by Tatiana Proskouriakoff and Charles R. Temple; *A Vaulted Temple at Mayapan* (no. 30), by Howard D. Winters; *Excavation of a Colonnaded Hall at Mayapan* (no. 31), by Howard D. Winters; *Three Serpent Column Temples and Associated Platforms at Mayapan* (no. 32), by Howard D. Winters.

Six other papers in this series are now in press. They are expected to appear during the summer and early autumn of 1956.

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- See THOMPSON, DONALD E.

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- Year Book No. 54, 1954-1955. Octavo, xxxix + 9 + 311 pages, 3 plates, 50 figures (1955).
608. A. LEDYARD SMITH. Archaeological reconnaissance in central Guatemala. Quarto, vii + 87 pages, frontispiece, 140 figures (1955).
609. ANNA O. SHEPARD. Ceramics for the archaeologist. Octavo, xii + 414 pages, 59 figures (1956).
610. PAUL W. MERRILL. Lines of the chemical elements in astronomical spectra. Quarto, vii + 167 pages, 2 figures, 36 diagrams (1956).

### PUBLICATIONS BY THE PRESIDENT OF THE INSTITUTION

#### VANNEVAR BUSH

An introduction. *In* The world we live in, by the editorial staff of *Life* and Lincoln Barnett, pp. 1-2. New York: Time, Inc. (1955).

Improved automatic microtome. (Letter) *Science*, vol. 122, no. 3159, p. 119 (July 15, 1955).

Statement of Dr. Vannevar Bush, President, Carnegie Institution of Washington. *In* Automation and technological change: Hearings before the Subcommittee on Economic Stabilization of the Joint Committee on the Economic Report, Congress of the United States, Eighty-fourth Congress, First Session . . . October 14, 15, 17, 18, 24, 25, 26, 27, and 28, 1955, pp. 604-617 (October 28) (1955).

#### Reprinted:

Scientific motivation (see Year Book No. 54).  
*Reprinted in slightly abridged form*, under the title "For man to know": *Atlantic Monthly*, vol. 196, no. 2, pp. 29-34 (Aug. 1955).

#### CARYL P. HASKINS

Spirit of scientific inquiry. (An address at the awards banquet of the Fifteenth Annual Science Talent Search, Washington, March 5.) *Science News Letter*, vol. 69, no. 10, pp. 154-158 (March 10, 1956).





## REPORT OF THE EXECUTIVE COMMITTEE

FOR THE YEAR ENDED JUNE 30, 1956

*To the Trustees of the Carnegie Institution of Washington:*

GENTLEMEN: In accordance with the provisions of the By-Laws, the Executive Committee submits this report to the annual meeting of the Board of Trustees.

During the fiscal year ended June 30, 1956, the Executive Committee held four meetings, printed reports of which have been mailed to each Trustee and constitute a part of this report.

The report of the President has been received by the Executive Committee and is presented with its approval, including the record of the scientific activities of the Departments, also contained in the Year Book. The estimate of expenditures for the calendar year 1957 has been reviewed by the Executive Committee.

The report of the Auditors, including a balance sheet showing assets and liabilities of the Institution on June 30, 1956, together with supporting statements and schedules, is submitted as a part of this report.

One vacancy exists in the membership of the Board of Trustees, resulting from the resignation of W. Cameron Forbes in December 1955.

The terms of Messrs. Bradford and Gifford as members of the Executive Committee will end at the annual meeting in December. Terms of Mr. Prentis as a member of the Finance Committee and of Mr. Morgan as a member of the Retirement Committee will end at the annual meeting. Terms of Mr. Bradford as Chairman of the Finance Committee, of Mr. McHugh as Chairman of the Auditing Committee, of Mr. Bradford as Chairman of the Retirement Committee, and of Mr. McHugh as Chairman of the Nominating Committee will end at the annual meeting. The term of Mr. McHugh as a member of the Nominating Committee will also end at the annual meeting.

BARKLIE MCKEE HENRY, *Chairman*

*October 25, 1956*





HASKINS & SELLS  
CERTIFIED PUBLIC ACCOUNTANTS

500 EQUITABLE BUILDING  
BALTIMORE 2

### ACCOUNTANTS' CERTIFICATE

*To the Board of Trustees of Carnegie Institution of Washington:*

We have examined the balance sheet of Carnegie Institution of Washington as of June 30, 1956 and the related summaries of current income and expenditures, current funds surplus, current restricted gifts and grants, changes in endowment and other special funds, and changes in investment in real estate and equipment for the year then ended (Exhibits A to F, inclusive). Our examination was made in accordance with generally accepted auditing standards, and accordingly included such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances.

In our opinion, the accompanying balance sheet and above described summaries (Exhibits A to F, inclusive) present fairly the financial position of the Institution at June 30, 1956 and the results of its operations for the year then ended, in conformity with generally accepted accounting principles applied on a basis consistent with that of the preceding year.

HASKINS & SELLS

*August 29, 1956*

## ASSETS

Current Funds:

Cash .....	\$625,393.21	
Advances:		
Departmental Research Operations .....	6,341.40	
Other .....	1,702.61	
Accounts receivable .....	601.09	
Deferred charges .....	41,322.94	
Due from Endowment and Other Special Funds .....	90,894.60	\$736,255.85

Endowment and Other Special Funds:

Cash .....	\$94,434.93	
Securities (valuation based on market quotations at June 30, 1956—\$74,303,376)—Schedule 1:		
Bonds .....	\$34,381,614.38	
Preferred stocks .....	3,765,300.75	
Common stocks .....	14,785,126.74	52,932,041.87
		53,026,476.80

Plant Funds:

Investment in real estate and equipment—Exhibit F .....	5,276,011.25
Total .....	\$59,068,743.90

## LIABILITIES

Current Funds:

Accounts payable .....	\$1,873.63	
Reserve for accounts receivable .....	601.09	
Current Funds Surplus—Exhibit C:		
Appropriated unexpended balances .....	\$494,728.32	
General Contingent Fund .....	205,982.72	700,711.04
Unexpended balance of restricted gifts and grants—Exhibit D .....	63,070.09	\$736,255.85

Endowment and Other Special Funds:

Due to Current Funds .....	\$90,894.60	
Principal of Funds—Exhibit E:		
Capital funds .....	\$49,344,354.62	
Special funds .....	3,591,227.58	52,935,582.20
		53,026,476.80

Plant Funds:

Bequests, gifts, and income invested in plant .....	\$5,232,511.25	
Harriman Fund—donated land .....	5,500.00	
Hale Fund—Solar Laboratory .....	38,000.00	5,276,011.25
Total .....		\$59,068,743.90

## EXHIBIT B

SUMMARY OF CURRENT INCOME AND EXPENDITURES  
FOR THE YEAR ENDED JUNE 30, 1956Current Income:

Investment income:		
Interest and dividends on securities .....	\$2,282,857.00	
Less: Amortization of bond premiums .....	25,328.98	\$2,257,528.02
Market value of stock dividend .....		7,950.00
Total—Schedule 1 .....		\$2,265,478.02
Less: Income added to Special Funds (Exhibit E)—Schedule 1 .....		4,845.83
Remainder—Income available for current purposes .....		\$2,260,632.19
Other income:		
Sales of publications .....	\$6,784.25	
Dormitory and mess hall .....	7,722.46	
Miscellaneous .....	13,592.29	28,099.00
Restricted gifts and grants utilized for current purposes—Exhibit D .....		81,688.42
Total Current Income .....		\$2,370,419.61

Current Expenditures (including expenditures against appropriations  
of prior years)—Schedule 4:

Administration .....	\$259,894.08	
Departmental Research Operations .....	1,435,519.28	
General Publications .....	28,274.15	
Research Projects, Fellowships, Grants, etc. ....	104,655.98	
Pension Fund—annuity and insurance .....	97,209.88	
Retirement Plan Contributions .....	141,126.40	
	\$2,066,679.77	
Gifts and grants—Exhibit D .....	81,688.42	
Total Current Expenditures .....		2,148,368.19
Excess of Current Income over Current Expenditures—Exhibit C .....		\$222,051.42 *

## \* Summarized as follows:

Current investment income in excess of Trustees' authorized appropriations during the fiscal year, credited to General Reserve Fund—Exhibit E .....	\$296,120.50	
Market value of stock dividend credited to Special Income Reserve— Exhibit E .....	7,950.00	
Transfer of unexpended current appropriations to General Contingent Fund—Schedule 4 .....	60,440.68	
Reserved from this year's appropriations for current liabilities and commitments—Schedule 4 .....	155,735.23	
Total .....	\$520,246.41	
Less: Amount included in current expenditures, applicable to allotments and unexpended balances from prior years' appropriations—Schedule 4 .....	298,194.99	\$222,051.42

## EXHIBIT C

SUMMARY OF CURRENT FUNDS SURPLUS  
FOR THE YEAR ENDED JUNE 30, 1956

Balance, July 1, 1955 .....		\$786,159.39
Additions:		
Excess of current income over current expenditures—Exhibit B .....	\$222,051.42	
Transfer from Special Funds—Exhibit E:		
Special Purpose Funds .....	5,000.00	227,051.42
		<hr/>
Total .....		\$1,013,210.81
Deductions:		
Transfer to Special Funds—Exhibit E:		
General Reserve Fund, representing excess of investment		
income over Trustees' appropriations for current		
purposes .....	\$296,120.50	
Special Income Reserve, representing market value of stock		
dividend received during year .....	7,950.00	
Special Purpose Funds—income .....	8,429.27	312,499.77
		<hr/>
Balance, June 30, 1956, per Schedule 3 .....		<u>\$700,711.04</u>



## EXHIBIT D

SUMMARY OF CURRENT RESTRICTED GIFTS AND GRANTS  
FOR THE YEAR ENDED JUNE 30, 1956

	Unexpended Balance July 1, 1955	Additions— Gifts and Grants Received	Deductions— Expenditures (Schedule 4)	Unexpended Balance June 30, 1956
Departmental Research Operations:				
Department of Genetics:				
American Cancer Society No. EG 21 .....	\$2,316.65	\$7,400.00	\$6,884.00	\$2,832.65
U. S. Public Health Service No. RG-149 C ..	3,664.81	9,573.39	10,515.61	2,722.59
U. S. Public Health Service No. C-2158 C ..	2,123.77	11,572.45	11,444.16	2,252.06
National Foundation for Infantile Paralysis .	13.57	.....	13.57	.....
Department of Terrestrial Magnetism:				
National Science Foundation .....	17,348.61	.....	5,832.01	11,516.60
National Science Foundation #2236 .....	.....	9,500.00	7,020.32	2,479.68
Department of Embryology:				
National Science Foundation #G2177 .....	.....	1,600.00	1,600.00	.....
U. S. Public Health Service - Greenwald ....	.....	500.00	469.79	30.21
U. S. Public Health Service - De Feo .....	.....	500.00	500.00	.....
Population Council Inc. ....	.....	14,850.00	5,500.00	9,350.00
Mount Wilson Observatory:				
Gift .....	.....	2,000.00	.....	2,000.00
Research Projects, Fellowships, etc.:				
Carnegie Corporation of New York:				
Biology:				
Point Lobos, California .....	2,383.07	.....	2,383.07	.....
Yerkes Laboratories of Primate Biology .....	.....	10,000.00	10,000.00	.....
Geology:				
Tilley, C. E. ....	620.63	.....	.....	620.63
Physiology:				
Russell, G. Oscar .....	1,222.44	.....	.....	1,222.44
Terrestrial Magnetism:				
Clews, C. J. Birkett .....	2,209.74	.....	2,051.10	158.64
Jeffery, Peter .....	2,627.50	.....	2,627.50	.....
Magnetic polarization of the earth .....	844.67	.....	844.67	.....
Telescope image converter .....	36,887.21	.....	14,002.62	22,884.59
Tuve, M. A., travel .....	.....	5,000.00	.....	5,000.00
Total .....	<u>\$72,262.67</u>	<u>\$72,495.84</u>	<u>\$81,688.42</u>	<u>\$63,070.09</u>

EXHIBIT E SUMMARY OF CHANGES IN ENDOWMENT AND OTHER SPECIAL FUNDS FOR THE YEAR ENDED JUNE 30, 1956

	Additions			Deductions		
	Balance July 1, 1955	Gifts, Sale of Property and Misc.	Income from Investments Added to Funds (Exhibit B)	Net Realized Gain on Investments (Schedule 2)	Transfer to Current Funds Surplus (Exhibit C)	Balance June 30, 1956
Capital Funds:						
Endowment Fund .....	\$32,000,000.00	.....	.....	.....	.....	\$32,000,000.00
Capital Reserve Fund .....	14,353,941.40	.....	.....	\$2,869,884.37	.....	17,223,825.77
Colburn Fund .....	103,310.80	.....	.....	.....	.....	103,310.80
Harkavy Fund .....	5,051.05	.....	.....	.....	.....	5,051.05
Teeple Fund .....	10,888.42	.....	.....	.....	.....	10,888.42
Van Gelder Fund .....	1,278.58	.....	.....	.....	.....	1,278.58
Special Funds:						
Bickel Fund .....	11,553.01	\$25.49	\$510.79	.....	.....	12,089.29
General Reserve Fund .....	2,945,180.57	2,467.55	296,120.50	.....	.....	3,243,768.62
George E. Hale Relief Fund .....	4,347.46	.....	189.76	.....	.....	4,537.22
Harkavy Fund—Income .....	1,049.71	.....	223.03	.....	.....	1,272.74
Harriet H. Mayor Relief Fund .....	6,066.67	.....	.....	.....	.....	4,516.67
Special Income Reserve .....	220,621.89	.....	7,950.00	.....	.....	228,571.89
Special Purpose Funds .....	62,250.00	15,579.27	3,051.52	.....	\$5,000.00	75,880.79
Woloff Fund .....	19,719.63	.....	870.73	.....	.....	20,590.36
Total .....	\$49,745,259.19	\$18,072.31	\$308,916.33	\$2,869,884.37	\$5,000.00	\$52,935,582.20

EXHIBIT F SUMMARY OF CHANGES IN INVESTMENT IN REAL ESTATE AND EQUIPMENT FOR THE YEAR ENDED JUNE 30, 1956

	Classification of June 30, 1956 Balance							
	Balance July 1, 1955	Additions (see Note)	Deductions	Balance June 30, 1956	Buildings and Grounds	Laboratory Apparatus	Library	Operating Equipment
Departments of Research:								
Department of Plant Biology Stanford, California .....	\$157,889.00	\$8,323.00	\$974.78	\$165,237.22	\$71,897.68	\$49,635.65	\$27,552.33	\$16,151.56
Department of Genetics Long Island, New York .....	1,172,616.35	8,114.51	3,156.89	1,177,573.97	985,150.41	81,723.31	76,439.29	34,260.96
Geophysical Laboratory Washington, D. C. ....	458,296.85	42,130.34	11,742.83	488,684.36	170,383.79	212,203.09	47,287.97	58,809.51
Department of Archaeology Cambridge, Massachusetts .....	18,324.13	.....	6,071.37	12,252.76	.....	.....	1,261.67	10,991.09
Mount Wilson Observatory Pasadena, California .....	1,665,059.16	5,999.39	.....	1,671,058.55	275,827.70	1,241,994.39	81,701.66	71,534.80
Department of Terrestrial Magnetism Washington, D. C. ....	809,565.65	32,519.43	4,273.20	837,811.88	401,418.69	313,663.30	47,008.46	75,721.43
Department of Embryology Baltimore, Maryland .....	57,661.46	22,570.82	.....	80,232.28	.....	63,228.28	9,670.31	7,333.69
Total Departments of Research ..	\$4,339,412.60	\$119,657.49	\$26,219.07	\$4,432,851.02	\$1,904,678.27	\$1,962,448.02	\$290,921.69	\$274,803.04
Office of Administration								
Washington, D. C. ....	835,512.23	7,648.00	.....	843,160.23	797,632.96	.....	.....	45,527.27
Total .....	\$5,174,924.83	\$127,305.49	\$26,219.07	\$5,276,011.25	\$2,702,311.23	\$1,962,448.02	\$290,921.69	\$320,330.31
Note: Additions during year provided from following:								
Current expenditures for equipment—Schedule 4 .....								\$117,846.77
Adjustment of capitalized equipment .....								1,158.72
Cost of constructed equipment capitalized:								
Department of Terrestrial Magnetism .....								8,300.00
Total, as above .....								\$127,305.49

## SCHEDULE 1

ENDOWMENT AND OTHER SPECIAL FUNDS INVESTMENTS AS OF  
JUNE 30, 1956 AND INCOME THEREFROM DURING THE YEAR

	Book Value	Market Value	Per Cent of Total Investments		Income Received
			Book Value	Market Value	
Bonds:					
United States Government .....	\$14,900,210.33	\$14,585,046	28.10%	19.60%	\$383,208.16
Foreign and International Bank .....	1,055,633.43	1,017,306	1.99%	1.37%	34,773.71
Public Utility .....	4,516,420.83	4,327,757	8.52%	5.82%	79,303.25
Communication .....	2,072,283.10	2,042,062	3.91%	2.74%	43,780.02
Railroad .....	473,435.16	492,670	.89%	.66%	20,762.53
Railroad Equipment Trust .....	977,359.88	962,910	1.84%	1.29%	23,708.16
Industrial and Miscellaneous .....	10,386,271.65	10,050,035	19.59%	13.51%	230,405.96
Total bonds .....	<u>\$34,381,614.38</u>	<u>\$33,477,786</u>	<u>64.84%</u>	<u>44.99%</u>	<u>\$815,941.79 *</u>
Stocks:					
Preferred .....	\$3,765,300.75	\$3,668,325	7.10%	4.93%	\$153,387.20
Common .....	14,785,126.74	37,157,265	27.88%	49.95%	1,296,149.03 †
Total stocks .....	<u>\$18,550,427.49</u>	<u>\$40,825,590</u>	<u>34.98%</u>	<u>54.88%</u>	<u>\$1,449,536.23</u>
Cash .....	<u>\$94,434.93</u>	<u>\$94,435</u>	<u>.18%</u>	<u>.13%</u>	<u>.....</u>
Total .....	<u>\$53,026,476.80</u>	<u>\$74,397,811</u>	<u>100.00%</u>	<u>100.00%</u>	<u>\$2,265,478.02 †</u>

\*After deducting bond premium amortization of \$25,328.98.

† Includes \$7,950.00 representing market value of a stock dividend received.

‡ Income received allocated to Endowment and Other Special Funds as follows:

Funds, the income from which may be used for current general purposes—Exhibit B .....	\$2,260,632.19
Funds, the income from which is restricted to specific purposes—Exhibit E:	
Bickel Fund .....	\$510.79
George E. Hale Relief Fund .....	189.76
Harkavy Fund .....	223.03
Special Purpose Funds .....	3,051.52
Woloff Fund .....	870.73
Total .....	<u><u>4,845.83</u></u>
Total .....	<u><u>\$2,265,478.02</u></u>



## SCHEDULE 2

## SCHEDULE OF SECURITIES

Principal amount	Description	Maturity	Book value	Approximate market value
United States Government Bonds				
\$225,000	U. S. of America Treasury Notes Series "A" 1½s .....	1959	\$224,929.69	\$219,516
675,000	U. S. of America Treasury Notes Series "C" 2s .....	1957	672,152.35	670,148
2,350,000	U. S. of America Treasury 2½s .....	1962-59	2,368,009.27 *	2,261,875
1,500,000	U. S. of America Treasury 2½s .....	1958	1,498,089.77	1,488,281
2,465,000	U. S. of America Treasury 2½s .....	1961	2,425,903.77	2,403,375
3,588,000	U. S. of America Treasury 2½s .....	1963	3,592,441.88 *	3,480,360
800,000	U. S. of America Treasury 2½s .....	1961	800,000.00	793,000
1,000,000	U. S. of America Treasury Series "B" 2½s .....	1980-75	991,093.75	941,875
1,325,000	U. S. of America Treasury 3s .....	1995	1,327,589.85 *	1,322,516
400,000	U. S. of America Treasury 3½s .....	1983-78	400,000.00	418,000
100,000	U. S. of America Savings Series "G" 2½s .....	1957	100,000.00	99,200
100,000	U. S. of America Savings Series "G" 2½s .....	1958	100,000.00	98,200
100,000	U. S. of America Savings Series "G" 2½s .....	1959	100,000.00	97,300
100,000	U. S. of America Savings Series "G" 2½s .....	1960	100,000.00	96,400
200,000	U. S. of America Savings Series "K" 2.76s .....	1966	200,000.00	195,000
<u>\$14,928,000</u>	Total U. S. Government .....		<u>\$14,900,210.33</u>	<u>\$14,585,046</u>
Foreign and International Bank Bonds				
\$250,000	Aluminum Company of Canada, Ltd., S. F. Deb. 3½s Guar. ..	1970	\$252,885.84 *	\$260,625
85,000	Australia, Commonwealth of, 4½s .....	1971	83,725.00	83,619
100,000	Canadian National Ry. Co., 4½s Guar. ....	1957	112,000.00	102,500
125,000	International Bank for Reconstruction and Development, 3s ..	1976	125,000.00	117,500
125,000	International Bank for Reconstruction and Development, 3½s.	1975	123,125.00	122,187
150,000	Noranda Mines Ltd., S. F. Deb. 4½s .....	1968	153,497.59 *	147,375
200,000	Shawinigan Water & Power Co., 1st Mtg. & Coll. Tr. S. F. Series "M" 3s .....	1971	205,400.00 *	183,500
<u>\$1,035,000</u>	Total Foreign and International Bank .....		<u>\$1,055,633.43</u>	<u>\$1,017,306</u>
Public Utility Bonds				
\$250,000	California Oregon Power Co., 1st Mtg. 3¼s .....	1986	\$253,337.50	\$251,250
125,000	Columbia Gas System, Inc., Series "B" 3s .....	1975	127,583.15 *	113,750
250,000	Columbia Gas System, Inc., Series "F" 3½s .....	1981	245,937.50	251,250
237,000	Columbus & Southern Ohio Electric Co., 1st Mtg. 3½s .....	1970	246,089.58 *	229,890
300,000	Commonwealth Edison Company 1st Mtg. Series "R" 3½s ...	1986	300,787.50	297,750
300,000	Consolidated Edison Co. of N. Y., 1st & Ref. Mtg. Series "L" 3½s .....	1986	304,131.00	301,500
300,000	Consolidated Natural Gas Co., Deb. 2½s .....	1968	300,508.78 *	283,500
200,000	Minnesota Power & Light Co., 1st Mtg. 3½s .....	1975	203,147.71 *	194,500
250,000	Niagara Mohawk Power Corp., Gen. Mtg. 3½s .....	1986	253,442.50	250,937
100,000	Ohio Power Co., 1st Mtg. 3½s .....	1968	101,500.00	99,750
200,000	Pacific Gas and Electric Co., 1st & Ref. Mtg. Series "X" 3½s	1984	201,633.32 *	192,000
300,000	Pacific Gas and Electric Co., 1st & Ref. Mtg. Series "Y" 3½s	1987	306,952.55 *	297,000
200,000	Panhandle Eastern Pipe Line Co., Serial Deb. 2½s .....	1961-62	201,047.69 *	189,250
87,000	Panhandle Eastern Pipe Line Co., S. F. Deb. 3½s .....	1973	88,065.22 *	82,650
50,000	Philadelphia Electric Co., 1st & Ref. Mtg. 2½s .....	1978	49,687.50	46,500
207,000	Philadelphia Electric Power Co., 1st Mtg. 2½s Guar. ....	1975	210,310.02 *	186,300
200,000	Public Service Co. of Indiana, Inc., 1st Mtg. Series "F" 3½s	1975	203,206.97 *	192,000
250,000	Southern California Edison Co., 1st & Ref. Mtg. Series "G" 3½s	1981	247,765.00	251,250
210,000	Tennessee Gas Transmission Co., 1st Mtg. Pipe Line 2½s ..	1966	211,575.00 *	195,300
191,000	Tennessee Gas Transmission Co., 1st Mtg. Pipe Line 3s ...	1969	194,712.34 *	177,630
265,000	United Gas Corp., 1st Mtg. & Coll. Tr. 2½s .....	1967	265,000.00	243,800
<u>\$4,472,000</u>	Total Public Utility .....		<u>\$4,516,420.83</u>	<u>\$4,327,757</u>
Communication Bonds				
\$150,000	American Telephone & Telegraph Co., Deb. 2½s .....	1975	\$151,631.25 *	\$136,312
350,000	American Telephone & Telegraph Co., Deb. 3½s .....	1984	362,036.88 *	336,875
600,000	American Telephone & Telegraph Co., Conv. Deb. 3½s .....	1967	747,136.98 *	791,250
200,000	Mountain States Telephone & Telegraph Co., Deb. 3½s .....	1978	201,190.00 *	193,500
100,000	New York Telephone Co., Ref. Mtg. Series "E" 3½s .....	1978	101,035.51 *	97,625
200,000	Pacific Telephone & Telegraph Co., Deb. 3½s .....	1978	203,752.48 *	194,750
300,000	Southwestern Bell Telephone Co., Deb. 3½s .....	1983	305,500.00 *	291,750
<u>\$1,900,000</u>	Total Communication .....		<u>\$2,072,283.10</u>	<u>\$2,042,062</u>

\*After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.

SCHEDULE OF SECURITIES—Continued

Principal amount	Description	Maturity	Book value	Approximate market value
<b>Railroad Bonds</b>				
\$100,000	Chesapeake & Ohio Ry. Co., Gen. Mtg. 4½s .....	1992	\$99,464.29	\$119,000
267,000	Fort Worth & Denver Rwy. Co., 1st Mtg. 4½s Guar. ....	1982	269,308.37 *	269,670
100,000	Pennsylvania R. R. Co., Cons. Mtg. 4½s .....	1960	104,662.50	104,000
<u>\$467,000</u>	<u>Total Railroad .....</u>		<u>\$473,435.16</u>	<u>\$492,670</u>
<b>Railroad Equipment Trust Bonds</b>				
\$100,000	Chesapeake & Ohio Ry. Co., Eq. Tr. 2s Guar. ....	1957-58	\$97,246.42	\$98,310
300,000	Chicago Burlington & Quincy R. R. Co., Eq. Tr. 2½s Guar. ..	1958-63	292,507.12	284,910
100,000	Great Northern Railway Co., Eq. Tr. 2s Guar. ....	1960-61	98,538.91	93,720
150,000	Pennsylvania R. R. Co., Eq. Tr. Series "S" 2½s Guar. ....	1958-62	146,358.96	142,740
100,000	Southern Pacific Co., Eq. Tr. Series "CC" 2½s Guar. ....	1956&59	99,718.17 *	97,550
100,000	Southern Pacific Co., Eq. Tr. Series "X" 2½s Guar. ....	1957-58	97,061.61	98,170
150,000	Southern Railway Co., Eq. Tr. Series "NN" 2½s Guar. ....	1956-58	145,928.69	147,510
<u>\$1,000,000</u>	<u>Total Railroad Equipment Trust .....</u>		<u>\$977,359.88</u>	<u>\$962,910</u>
<b>Industrial and Miscellaneous Bonds</b>				
\$200,000	Allied Chemical and Dye Corp., Deb. 3½s .....	1978	\$198,000.00	\$204,250
200,000	Aluminum Company of America, S. F. Deb. 3½s .....	1964	200,000.00	199,750
100,000	Aluminum Company of America, S. F. Deb. 3s .....	1979	100,000.00	95,250
187,000	American Tobacco Co., Deb. 3s .....	1969	188,773.45 *	180,155
234,000	Bristol Myers Co., Deb. 3s .....	1968	234,683.38 *	224,640
300,000	C. I. T. Financial Corp., Deb. 2½s .....	1959	300,000.00 *	291,000
400,000	Commercial Credit Co., Notes, 3½s .....	1976	409,940.00	400,000
400,000	Continental Oil Company, S. F. Deb. 3s .....	1984	404,741.40 *	380,500
150,000	Dow Chemical Co., Deb. 2.35s .....	1961	150,306.83 *	142,500
130,000	Dow Chemical Co., Conv. Sub. Deb. 3s .....	1982	131,727.30 *	214,500
435,000	Federal Home Loan Banks, Cons. Notes Series "H" 3½s ....	1956	435,135.94	435,000
153,000	Food Machinery Corp., S. F. Deb. 2½s .....	1962	152,308.98	144,585
500,000	General Electric Co., Deb. 3½s .....	1976	502,500.00	507,500
250,000	General Motors Acceptance Corp., Deb. 3s .....	1960	250,000.00	245,937
200,000	General Motors Acceptance Corp., Deb. 3½s .....	1972	204,909.10 *	198,750
180,000	General Motors Acceptance Corp., Deb. 4s .....	1958	180,000.00	182,475
500,000	General Motors Corporation, Deb. 3½s .....	1979	502,250.00 *	496,875
275,000	Goodrich (B. F.) Company, 1st Mtg. 2½s .....	1965	275,579.01 *	264,000
500,000	Illinois State Toll Highway Comm., Rev. Bonds 3½s .....	1995	500,000.00	467,500
236,000	P. Lorillard Co., Deb. 3s .....	1963	239,253.02 *	228,920
295,000	National Dairy Products Corp., Deb. 2½s .....	1970	297,851.58 *	277,300
488,000	Phillips Petroleum Co., S. F. Deb. 2½s .....	1964	491,422.79 *	468,480
125,000	Pittsburgh Plate Glass Co., S. F. Deb. 3s .....	1967	125,000.00	123,125
150,000	Quaker Oats Co., Deb. 2½s .....	1964	148,922.50	142,875
100,000	Riegel Paper Corp., S. F. Deb. 3½s .....	1981	100,000.00	100,250
300,000	Seagram (Joseph E.) & Sons, Inc., Deb. 2½s .....	1966	298,500.00	276,000
300,000	Service Pipe Line Co., S. F. Deb. 3.20s .....	1982	300,000.00	292,500
500,000	Shell Union Oil Corp., Deb. 2½s .....	1971	502,978.79 *	460,625
500,000	Socony Mobil Oil Co., Inc., Deb. 2½s .....	1976	489,528.75	457,500
300,000	Swift & Co., Deb. 2½s .....	1972	301,126.33 *	279,000
500,000	Texas Corporation, Deb. 3s .....	1965	514,287.67 *	496,250
250,000	Tide Water Associated Oil Co., S. F. Deb. 3½s .....	1986	250,000.00	245,625
346,000	Union Oil Company of California, Deb. 2½s .....	1970	353,567.59 *	319,618
400,000	Westinghouse Electric Corporation, Deb. 2½s .....	1971	402,977.24 *	364,000
250,000	Whirlpool-Seeger Corp., S. F. Deb. 3½s .....	1980	250,000.00	242,500
<u>\$10,334,000</u>	<u>Total Industrial and Miscellaneous .....</u>		<u>\$10,386,271.65</u>	<u>\$10,050,035</u>
<u>\$34,136,000</u>	<u>Bonds—Funds Invested .....</u>		<u>\$34,381,614.38</u>	<u>\$33,477,786</u>

\* After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.

SCHEDULE OF SECURITIES—Continued

Number of shares	Description	Book value	Approximate market value
<b>Preferred Stocks</b>			
1,500	Appalachian Electric Power Co., 4½% Cum. Pref. ....	\$159,000.00	\$160,687
2,000	Armstrong Cork Co., \$3.75 Cum. Pref. ....	205,500.00	192,000
1,500	Bethlehem Steel Corp., 7% Cum. Pref. ....	183,637.50	244,500
3,800	Carrier Corporation, 4½% Cum. Pref. ....	197,931.28	183,350
500	Case (J. I.) Co., 7% Cum. Pref. ....	62,225.00	51,500
600	Cleveland Electric Illuminating Co., \$4.50 Cum. Pref. ....	68,112.25	64,875
1,900	Consolidated Edison Co. of N. Y., Inc., \$5.00 Cum. Pref. ....	202,815.50	209,000
1,125	Continental Can Co., Inc., \$3.75 Cum. Pref. ....	115,312.50	110,250
600	Corn Products Refining Co., 7% Cum. Pref. ....	110,335.18	103,200
2,075	duPont (E. I.) de Nemours & Co., \$4.50 Cum. Pref. ....	235,401.89	244,331
1,000	El Paso Natural Gas Co., 4.10% Cum. Pref. ....	111,442.21	92,000
1,500	General Motors Corp., \$5.00 Cum. Pref. ....	187,937.50	181,875
1,000	General Shoe Corporation, \$3.50 Cum. Pref. Series "A" ....	102,250.00	87,000
1,000	Grant (W. T.) Co., 3¾% Cum. Pref. ....	100,447.91	92,500
300	Merck & Co., Inc., \$4.00 Cum. Conv. 2nd Pref. ....	31,200.00	33,600
800	National Distillers Products Corp., 4¼% Cum. Conv. Pref. ....	80,000.00	78,600
2,000	Niagara Mohawk Power Corp., 3.60% Cum. Pref. ....	207,990.00	167,000
1,300	Ohio Power Co., 4½% Cum. Pref. ....	144,630.02	141,375
1,500	Pacific Telephone and Telegraph Co., 6% Cum. Pref. ....	235,220.75	216,375
1,000	Panhandle Eastern Pipe Line Co., 4% Cum. Pref. ....	104,166.68	95,000
673	Pillsbury Mills, Inc., \$4.00 Cum. Pref. ....	72,496.91	66,627
2,000	Reynolds (R. J.) Tobacco Co., 3.60% Cum. Pref. ....	199,683.75	171,000
680	Sherwin-Williams Co., 4% Cum. Pref. ....	74,947.48	68,680
1,200	Standard Oil Co. of Ohio, 3¾% Cum. Pref. Series "A" ....	129,208.87	117,000
3,100	U. S. Steel Corp., 7% Cum. Pref. ....	443,407.57	496,000
<u>34,653</u>	<b>Total Preferred Stocks</b> .....	<u>\$3,765,300.75</u>	<u>\$3,668,325</u>
<b>Common Stocks</b>			
2,100	Allied Chemical & Dye Corporation .....	\$96,175.97	\$236,250
5,830	Aluminium Limited .....	247,080.22	792,880
5,000	Aluminum Company of America .....	208,437.50	598,750
15,682.5	American Gas and Electric Company .....	214,294.48	625,340
5,000	American Telephone & Telegraph Co. ....	737,124.15	900,000
8,000	Armco Steel Corporation .....	300,711.62	464,000
14,100	Armstrong Cork Company .....	231,516.80	477,637
2,800	Atchison, Topeka and Santa Fe Rwy. Co. ....	232,758.69	439,950
4,000	Bethlehem Steel Corporation .....	297,908.79	605,500
3,500	C. I. T. Financial Corporation .....	70,638.96	158,375
5,000	Carrier Corporation .....	202,162.77	278,750
4,000	Caterpillar Tractor Company .....	96,913.60	338,000
2,750	Cerro de Pasco Corporation .....	155,366.87	171,875
2,500	Chase Manhattan Bank of New York .....	71,361.04	122,187
60	Christiana Securities Co. ....	356,143.00	882,000
100	Chrysler Corporation .....	6,850.00	6,462
4,200	Consumers Power Co. ....	145,974.04	198,975
4,600	Continental Can Company, Inc. ....	106,779.99	218,500
6,000	Continental Insurance Co. ....	113,787.87	289,500
12,200	Continental Oil Co. of Delaware .....	239,598.64	1,509,750
2,500	Corning Glass Works .....	59,631.83	204,062
6,100	Delaware Power & Light Company .....	128,803.87	260,012
1,284	Dow Chemical Company .....	154,131.30	327,190
3,800	duPont (E. I.) de Nemours & Co. ....	155,091.50	801,800
8,379	Eastman Kodak Company .....	209,388.59	737,726
5,700	First National City Bank of New York .....	279,775.25	359,812
6,000	Florida Power & Light Company .....	148,863.69	267,750

(Concluded on following page)



SCHEDULE OF SECURITIES—Continued

Number of shares	Description	Book value	Approximate market value
<u>Common Stocks—Concluded</u>			
2,500	Ford Motor Company .....	\$161,250.00	\$141,250
34,000	General Electric Company .....	701,178.76	2,074,000
8,000	General Foods Corporation .....	167,302.84	373,000
18,000	General Motors Corporation .....	332,709.67	810,000
7,100	Goodrich (B. F.) Company .....	208,198.68	582,200
5,000	Goodyear Tire & Rubber Company of Ohio .....	323,584.14	361,875
1,800	Great Northern Paper Company .....	174,190.91	181,800
11,440	Gulf Oil Corp. ....	218,016.33	1,435,720
10,625	Gulf States Utilities Co. ....	223,782.34	377,187
2,500	Halliburton Oil Well Cementing Company .....	47,815.65	197,187
266.66	Hevi-Duty Electric Co. ....	2,884.42	4,467
2,500	Illinois Power Co. ....	97,697.35	142,500
7,200	Insurance Company of North America .....	153,326.32	637,200
2,500	International Business Machines Corp. ....	93,469.61	1,223,750
5,000	International Nickel Co. of Canada, Ltd. ....	185,533.15	496,250
4,200	International Paper Company .....	189,122.23	563,850
6,500	Island Creek Coal Company .....	284,937.90	302,250
3,200	Johns-Manville Corp. ....	83,825.34	162,400
3,900	Kennebec Copper Corporation .....	199,127.15	485,550
8,640	Kimberly-Clark Corporation .....	182,251.11	442,800
9,400	Lehigh Portland Cement Company .....	278,294.96	428,875
1,280	Mellon National Bank and Trust Company .....	67,193.07	138,240
5,000	Merck & Co., Inc. ....	93,798.41	167,500
9,000	Minneapolis-Honeywell Regulator Co. ....	103,971.05	735,750
12,000	Monsanto Chemical Co. ....	256,904.10	525,000
2,700	Northwest Bancorporation .....	187,854.24	189,675
3,300	Ohio Edison Co. ....	105,150.00	173,250
2,400	Phelps Dodge Corporation .....	71,057.69	147,300
1,990	Pittsburgh Plate Glass Co. ....	61,151.17	170,145
8,400	Procter & Gamble Co. ....	177,227.28	410,550
15,000	Puget Sound Power and Light Company .....	367,935.80	414,375
5,600	Scott Paper Co. ....	74,258.77	397,600
7,800	Seaboard Oil Co. ....	229,104.22	467,025
12,180	Sears, Roebuck & Co. ....	91,775.90	376,057
11,220	Shell Oil Company .....	413,016.26	908,820
12,375	Socony Mobil Oil Company, Inc. ....	300,464.13	716,203
5,800	Southern California Edison Company .....	208,276.33	297,975
11,250	Southern Railway Co. ....	218,508.81	511,875
8,000	Standard Oil Co. of Indiana .....	145,038.34	481,000
30,165	Standard Oil Co. of New Jersey .....	249,661.18	1,723,176
8,400	Texas Company .....	110,850.60	541,800
5,600	Texas Utilities Company .....	154,501.13	224,000
2,700	Union Carbide & Carbon Corp. ....	84,640.89	338,512
8,000	Union Electric Company of Missouri .....	137,750.63	217,000
1,000	Union Pacific R. R. Co. ....	114,547.42	172,875
8,855	United Gas Corp. ....	144,892.59	264,543
10,000	United States Gypsum Co. ....	181,208.10	665,000
18,100	United States Steel Corporation .....	413,722.19	1,045,275
6,000	Virginia Electric and Power Co. ....	130,447.67	261,000
8,000	West Virginia Pulp and Paper Co. ....	226,533.72	480,000
6,800	Weyerhaeuser Timber Company .....	87,917.16	268,600
<u>547,372.16</u>	<u>Total Common Stocks .....</u>	<u>\$14,785,126.74</u>	<u>\$37,157,265</u>
	<u>Common and Preferred Stocks—Funds Invested .....</u>	<u>\$18,550,427.49</u>	<u>\$40,825,590</u>
	<u>Aggregate Investments (Bonds and Stocks) .....</u>	<u>\$52,932,041.87</u>	<u>\$74,303,376</u>



SCHEDULE OF SECURITIES—Concluded

SUMMARY OF SECURITY TRANSACTIONS JULY 1, 1955 TO JUNE 30, 1956

Cash awaiting investment—July 1, 1955 .....	\$50,506.11
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Sales and Redemptions

	Gain	Loss	Book Value	
Bonds .....	\$117,050.43	.....	\$5,267,829.86	
Preferred Stocks .....	9,625.22	.....	217,500.10	
Common Stocks .....	2,723,286.43	.....	1,830,345.62	
Sale of Stock Rights .....	19,305.11	.....	.....	
Mortgages .....	617.18	.....	.....	
	<u>\$2,869,884.37</u>	<u>.....</u>	<u>\$7,315,675.58</u>	
Net Gain—To Exhibit E .....	.....	\$2,869,884.37	2,869,884.37	10,185,559.95
	<u>\$2,869,884.37</u>	<u>\$2,869,884.37</u>		

Income applied to amortization of bond premiums .....	25,328.98
Gift of 100 shares Chrysler Corporation common stock .....	6,850.00
Market value of stock dividend .....	7,950.00
Surplus cash transferred for investment .....	300,000.00
<b>Total</b> .....	<u>\$10,576,195.04</u>

Acquisitions

Bonds .....	\$8,981,807.87	
Common Stocks .....	1,302,020.96	
Preferred Stocks .....	197,931.28	10,481,760.11
Cash awaiting investment—June 30, 1956 .....		<u>\$94,434.93</u>

**SCHEDULE 3 SUMMARY OF CHANGES IN CURRENT FUNDS SURPLUS ACCOUNTS AND RESTRICTED GIFTS AND GRANTS  
FOR THE YEAR ENDED JUNE 30, 1956**

	Balance July 1, 1955	Additions			Deductions			Balance June 30, 1956
		Trustees' Appropriations (Schedule 4)	Allotments and Transfers—Net	Other Credits	Transfer From Special Funds	Expenditures (Schedule 4)	Transfers to General Contingent Fund (see Note)	
Current Funds Surplus:								
Appropriated:								
Departmental Research Operations:								
Department of Plant Biology .....	\$11,376.72	\$107,050.00	\$4,766.07	.....	.....	\$105,800.15	\$13,379.49	\$4,013.15
Department of Genetics .....	4,994.30	177,026.00	2,525.00	.....	.....	176,168.99	6,760.93	1,615.38
Dormitory and Mess Hall .....	2,266.24	1,200.00	.....	\$7,722.46	.....	9,329.55	.....	1,859.15
Geophysical Laboratory .....	36,412.38	214,900.00	71,935.07	.....	.....	296,822.87	2,924.65	23,499.93
Department of Archaeology .....	2,912.45	81,800.00	.....	.....	.....	69,485.64	13,317.85	1,908.96
Mount Wilson Observatory .....	24,370.82	287,250.00	3,275.10	.....	.....	274,995.14	13,144.56	26,756.22
Department of Terrestrial Magnetism .....	29,164.21	323,320.00	14,052.52	10,125.00	.....	351,170.46	1,736.40	23,754.87
Department of Embryology .....	8,453.53	132,885.00	42,461.08	.....	.....	151,746.48	7,919.44	24,133.69
Total Departmental Research Operations ..	\$119,950.65	\$1,325,431.00	\$139,014.84	\$17,847.46	.....	\$1,435,519.28	\$59,183.32	\$107,541.35
Administration .....	9,643.12	226,925.00	25,375.39	.....	\$5,000.00	259,894.08	1,757.92	5,291.51
General Operations .....	67,231.53	156,250.00	—152,790.52	1,082.89	—1,794.31	.....	.....	69,979.59
General Publications .....	61,363.35	30,000.00	.....	6,784.25	.....	28,274.15	.....	69,873.45
Research Projects, Fellowships, etc. ....	92,618.55	.....	127,067.30	.....	.....	104,655.98	5,280.49	109,749.38
Pension Fund .....	138,230.31	79,756.96	5,332.99	2,384.40	.....	97,209.88	939.95	127,554.83
Retirement Plan Contributions .....	781.52	139,983.04	5,090.05	.....	.....	141,126.40	.....	4,738.21
Total .....	\$489,819.03	\$1,958,356.00	\$149,090.05	\$28,099.00	\$3,205.69	\$2,066,679.77	\$67,161.68	\$494,728.32
Unallotted:								
General Contingent Fund .....	296,340.36	.....	—149,090.05	.....	—8,429.27	.....	—67,161.68	205,982.72
Total Current Funds Surplus— Exhibit C .....	\$786,159.39	\$1,958,356.00	.....	\$28,099.00	—\$5,223.58	\$2,066,679.77	.....	\$700,711.04
Restricted Gifts and Grants—Exhibit D .....	72,262.67	.....	.....	72,495.84	.....	81,688.42	.....	63,070.09
Total .....	\$858,422.06	\$1,958,356.00	.....	\$100,594.84	—\$5,223.58	\$2,148,368.19	.....	\$763,781.13
Note: Transfers to General Contingent Fund:								
From Current Year's Appropriations—Schedule 4 .....								\$60,440.68
From Prior Years' Appropriations .....								6,721.00
Total .....								\$67,161.68

SCHEDULE 4 STATEMENT OF EXPENDITURES AND BUDGET SUMMARY INCLUDING MISCELLANEOUS RECEIPTS  
FOR THE YEAR ENDED JUNE 30, 1956

	Expenditures Against			
	Current Year's Appropriations and Other Credits	Total Expenditures	Prior Years' Appropriations	Prior Appropriations
Departmental Research Operations:				
Department of Plant Biology .....	\$78,221.87	\$20,414.00	\$105,800.15	\$15,222.40
Department of Genetics .....	131,915.81	36,138.67	176,168.99	4,224.38
Dormitory and Mess Hall .....	3,539.00	5,790.55	9,328.55	2,266.24
Geophysical Laboratory .....	171,022.65	83,669.88	286,822.87	65,531.80
Department of Archaeology .....	55,781.17	13,704.47	69,485.64	2,299.18
Mount Wilson Observatory .....	213,862.77	55,132.98	274,995.14	24,995.92
Department of Terrestrial Magnetism .....	254,893.81	24,219.43	351,170.46	23,681.00
Department of Embryology .....	98,569.60	22,570.82	151,746.48	32,174.31
Total Departmental Research Operations .....	\$1,007,806.68	\$317,513.83	\$1,435,519.28	\$170,395.23
Administration .....	151,412.26	108,481.82	259,894.08	16,187.02
General Publications .....	.....	7,648.00	28,274.15	18,604.15
Research Projects, Fellowships, etc. ....	2,260.00	52,927.16	104,655.98	69,684.10
Pension Fund .....	.....	97,209.88	97,209.88	17,452.92
Retirement Plan Contributions .....	.....	141,126.40	141,126.40	5,871.57
Total .....	\$1,161,478.94	\$737,885.24	\$2,066,679.77	\$298,194.99
Restricted Gifts and Grants (see contra income—Exhibit B) .	33,519.98	45,540.94	81,688.42	.....
Total .....	\$1,194,998.92	\$783,426.18	\$2,148,368.19	.....
Budgets approved by Trustees:				
Appropriations July 1 to December 31, 1955 .....	.....	\$955,836.00	.....	.....
Appropriations January 1 to June 30, 1956 .....	.....	1,002,520.00	.....	.....
Total Appropriations—Schedule 3 .....	.....	\$1,958,356.00	.....	.....
Other Credits—Exhibit B:				
Sales of publications .....	.....	\$6,784.25	.....	.....
Proceeds from Dormitory and Mess Hall, Cold Spring Harbor .....	.....	7,722.46	.....	.....
Miscellaneous .....	.....	13,592.29	28,099.00	.....
Transfer of unexpended current appropriations to General Contingent Fund—Exhibit B .....	.....	.....	.....	60,440.68
Reserved from this year's appropriations for current liabilities and commitments—Exhibit B .....	.....	.....	.....	155,735.23
Income added to Special Funds—Exhibit B .....	.....	\$4,845.83	.....	.....
Less: Income (not budgeted) .....	.....	3,051.52	.....	1,794.31
Total .....	.....	\$1,986,455.00	\$1,986,455.00	.....





## ABSTRACT OF MINUTES OF THE FIFTY-EIGHTH MEETING OF THE BOARD OF TRUSTEES

The annual meeting of the Board of Trustees was held in Washington, D. C., on Friday, December 14, 1956. The Chairman, Mr. Root, presided.

The following Trustees were in attendance: James F. Bell, Robert Woods Bliss, Lindsay Bradford, Omar N. Bradley, Walter S. Gifford, Caryl P. Haskins, Barklie McKee Henry, Ernest O. Lawrence, Alfred L. Loomis, Robert A. Lovett, Keith S. McHugh, Margaret Carnegie Miller, Henry S. Morgan, William I. Myers, Henning W. Prentis, Jr., Elihu Root, Jr., Henry R. Shepley, Charles P. Taft, and Juan T. Trippe.

The minutes of the fifty-eighth meeting were approved.

In accordance with the recommendation of the Nominating Committee, James Norman White was elected a member of the Board of Trustees.

The Chairman announced the resignation of David Rockefeller as a Trustee. His resignation was accepted with regret.

Reports of the President, the Executive Committee, the Finance Committee, the Retirement Committee, the Auditor, the Auditing Committee, and the Nominating Committee, and of the Directors of Departments and Research Associates of the Institution were presented.

The sum of \$2,124,000 was appropriated for the calendar year 1957 for expenditure under the general charge and control of the Executive Committee.

In presenting the recommendations of the Nominating Committee, Chairman McHugh included with regret the advice that Elihu Root, Jr. had requested that he be relieved as Chairman of the Board of Trustees. The Board elected Walter S. Gifford as Chairman for the remainder of Mr. Root's term of office. Lindsay Bradford was re-elected a member of the Executive Committee for a three-year term, and Robert A. Lovett was elected a member of the Executive Committee for a three-year term. Lindsay Bradford and Henning W. Prentis, Jr. were re-elected members of the Finance Committee for three-year terms, and James Norman White was elected a member of the Finance Committee for a three-year term as an additional member increasing the membership of the Finance Committee from five to six members. Alfred L. Loomis was elected a member of the Auditing Committee for the remainder of the term of David Rockefeller. Walter S. Gifford upon election as Chairman of the Board became ex-officio member of the Nominating Committee replacing Mr. Root as ex-officio member, and Mr. Root was elected a member for a period of three years, succeeding Mr. McHugh. Henry S. Morgan was re-elected a member of the Retirement Committee. The following were elected for one-year terms: Barklie McKee Henry as Chairman of the Executive Committee, Lindsay Bradford as Chairman of the Finance Committee and as Chairman of the Retirement Committee, Keith S. McHugh as Chairman of the Auditing Committee, and Elihu Root, Jr. as Chairman of the Nominating Committee.



# INDEX

(Lower-case Roman numerals refer to front matter; capitals, to back matter.)

## A

- Abell, George Ogden, 60  
 studies in astronomy, 33, 37, 49-50  
 publication by, 60
- Abelson, Philip H., ix, 221, 222  
 report of Director of Geophysical Laboratory, 153-229  
 studies in paleobiochemistry, 15, 153, 171-174
- Acuña, Raul, 341
- Adams, Leason H., ix
- Adams, Mark, 296
- Adams, Walter S., ix, 22-23, 59  
 publications by, 60
- Administration, Office of, xi
- Agassiz, Alexander, vii, 3
- age of rocks, studies on, 13-14, 71, 87-100, 153, 161-168
- Ahrens, L. H., publication by, 229
- Ahrne, Ingrid M., 234
- Albright, J. F., 272  
 studies in embryology, 275
- Aldrich, L. Thomas, ix, 152, 220, 221  
 studies on age of rocks, 87-100, 161-168, 227  
 publications by, 150, 152, 228, 229
- Alexander, A. F. O'D., 74
- algae, studies on, 232-233, 259-265
- Allen, E. T., 190
- Allen, Willard, 27, 282
- Aller, L. H., 42, 55  
 publication by, 60
- Alter, Dinsmore, 55
- Aly, M. K., 44, 55  
 publication by, 60, 63
- Ambler, E., publication by, 150
- American Geophysical Union, Transactions of*, 24
- amphiboles, studies on, 169-171, 198-200
- anatomy, *see* embryology
- Anderson, Charles A., 220
- anhydrous silicate systems, *see* silicates, studies on
- archaeology, x  
 Committee on, vi  
 studies in, 22, 335-342
- Archaeology, Department of, x, 22, 25, 28  
 report of Director of Department of, 335-342
- Arnold, Ralph G., geophysical investigations, 177-179
- Aronson, John F., 317, 318
- Arp, Halton C., 40  
 publications by, 60-61
- astronomy, ix  
 Committee on, vi  
 studies in, 10-13, 31-63  
*See also* Image Tubes for Telescopes, report of Joint Committee on; radio astronomy
- astronomy, radio, *see* radio astronomy
- astrophysics, *see* astronomy
- Auditing Committee, vi, I, XIX
- Auditor, I, XIX  
 report of, III-XVII

## B

- Baade, Walter, ix, 31, 37, 59  
 stellar and nebular investigations, 11-12, 13, 31, 45, 47-48, 49, 51, 57, 59  
 publications by, 61
- Babcock, Harold D., solar research, 35
- Babcock, Horace W., ix, 31, 59  
 stellar research, 43, 53, 54  
 publications by, 61
- Bacher, Robert S., 31
- bacteria, *see* bacterial genetics; biophysics
- bacterial genetics, studies in, 16, 17, 294-295, 301-315
- bacteriophage, studies on, 15-16, 294, 297-301
- Baer, Harold, 295-296, 302  
 studies in bacterial genetics, 306-307
- Bailey, Irving M., 236
- Baldwin, George J., vii
- Bappu, M. K. V., 44
- Barbour, Thomas, vii
- Barnes, H. L., 221
- Barnett, S. J., 72
- Bartelmez, George W., x  
 studies in embryology, 19-20, 284, 285, 289  
 publications by, 291
- Bartels, J., 100
- Barth, Tom. F. W., 190
- Bartholome, P., 222
- Bauer, Ailene J., xi
- Bauer, Louis A., ix
- Baum, William A., ix, 60, 65, 66  
 nebular and stellar research, 13, 39, 40, 48, 49, 50, 51, 54  
 publications by, 61
- Beach, Alice S., 59, 60
- Beadle, George W., 236
- Beard, Robert G., 271
- Bell, James F., v, vi, XIX
- Benitez, A., publication by, 267
- Berkebile, Wilma J., publication by, 61
- Berlin, Heinrich, 338  
 publications by, 341
- Berlin, T., 222
- Berman, Robert, 340
- Bernheimer, Alan, 296
- Bernstein, M., publication by, 332
- Biesecker, Earle B., xi
- Billings, John S., vii
- Billings, M. P., 220
- biochemical investigations:  
 (embryology), 18, 19, 20, 27, 269, 271, 272, 273-275, 277-279, 280-281, 282-283, 285-288  
 (genetics), 16, 17, 294, 303-313, 316, 319, 320-322  
 (plant biology), 232, 243-265  
*See also* biophysics; paleobiochemistry
- biological sciences, x  
 Committee on, vi  
 studies in, 15-22, 231-333  
*See also* biophysics

- biophysics, studies in, 15, 72, 110-148, 154  
*See also* biochemical investigations; genetics; paleo-biochemistry
- Birch, Francis, 218, 219
- Bishop, David W., x, 271  
 studies in embryology, 19, 279, 280-281, 290  
 publication by, 291, 292
- Blakeslee, Albert F., x
- Bliss, Robert Woods, v, vi, XIX
- Bohr, A., 71, 104, 105
- Boise, James W., xi
- Bolton, Ellis T., ix, 152, 305  
 studies in biophysics, 110-148
- Bolton, J. G., 52
- Bonsack, Walter K., publication by, 61
- Botanical Research, Department of, x
- botany, *see* plant biology; paleobotany
- Böving, Bent G., x, 290  
 studies in embryology, 18-19, 272, 277, 285  
 publication by, 291
- Bowen, Ira S., ix, 28, 60  
 report of Director of Mount Wilson and Palomar Observatories, 31-63  
 publications by, 60, 61
- Bowen, Norman Levi, 25-26, 220  
 geophysical investigations, 182, 186, 190, 191, 209-210, 211, 212, 213, 214, 218, 224, 225-226, 227-228  
 publications by, 228
- Boyd, Francis R., Jr., ix, 221  
 geophysical investigations, 153, 154-157, 198-200, 208-210, 224  
 publication by, 228
- Boyden, Alan A., 147
- Bradford, Lindsay, v, vi, I, XIX
- Bradley, Omar N., v, XIX
- Briggs, Winslow, 242
- Britten, Roy J., ix, 82, 152, 305  
 studies in biophysics, 110-148  
 publication by, 150
- Brodführer, Ursula, x, 234, 235
- Brookings, Robert S., vii
- Brown, H., publications by, 150, 151, 229
- Buchanan, J. M., 296
- Buddington, A. F., 219, 220
- Burbidge, E. Margaret, stellar research, 40, 42, 45, 52  
 publication by, 61
- Burbidge, Geoffrey R., 60  
 stellar research, 40, 42, 43, 45, 52, 53  
 publications by, 61
- Burd, Sylvia, 60
- Burgi, Elizabeth, x  
 studies on bacteriophage, 297-301
- Burke, Bernard F., ix, 152  
 studies in radio astronomy, 73-78  
 upper atmosphere research, 80-81  
 publications by, 150
- Burns, Robert K., x, 28, 289-290  
 studies in embryology, 277-278  
 publications by, 291
- Bush, Vannevar, vii, 28, 65, 148, 217-219, 233, 236  
 publications by, 343  
*See also* Vannevar Bush Symposium
- Callaway, Samuel, xi
- Campbell, William W., vii
- Carnegie, Andrew, 3, 70
- Carnegie Collection of Embryos, 275, 276, 290
- Carnegie Corporation of New York, 65
- Carty, John J., vii
- Caspari, E. W., 296
- cellular organization, studies on:  
 (biophysics), 15, 72, 110-145  
 (genetics), 15-16, 17, 18, 294, 315-322
- ceramics, *see* archaeology
- Chamberlain, J. W., 42
- Chaney, Ralph W., 235  
 studies in paleobotany, 265-266
- Chayes, Felix, ix, 221, 222  
 studies on feldspars, 195-197  
 studies in statistical petrology, 210-216, 224  
 publication by, 228
- chemistry, *see* biochemical investigations; geophysical investigations; paleobiochemistry
- Chen, W. T., 222  
 geophysical investigations, 224, 225  
 publications by, 228
- Chinard, Francis P., studies in embryology, 285-286  
 publications by, 291, 292
- chloritoid, studies on, 200-203
- Christy, R. F., 45
- Clark, S., 222
- Clausen, Jens C., x, 234, 235  
 studies in experimental taxonomy, 26-27, 234, 236-239
- Clausen, Roy E., 236
- Clench, W. J., 340
- Clowes, Royston C., x, 295, 302  
 studies in bacterial genetics, 301, 304-306, 310, 311-312
- Code, Arthur, 55
- Coffeen, Mary F., 45, 60
- Cold Spring Harbor Symposium on Quantitative Biology, 293, 296, 315
- Cole, Whiteford R., vii
- Contributions to American Anthropology and History*, 341
- Contributions to Embryology*, 275, 276, 277, 288, 291
- Cope, Mary McCarthy, 270, 276
- Corner, George Washington, x, 27-28, 269-270, 278, 279, 284, 285, 290  
 studies in embryology, 272, 275, 282, 286  
 publications by, 267, 291
- cosmic-ray investigations, 101-103
- Cowie, Dean B., ix, 152  
 studies in biophysics, 110-148  
 publication by, 150
- Cox, Lois M., 234, 238
- Cragg, Thomas A., 60  
 solar research, 34
- Craig, Harmon B., 217-218, 219
- Creaser, E. H., ix, 152  
 studies in biophysics, 110-148
- Crowder, Roy E., 272  
 studies in embryology, 275  
 publication by, 291
- crystallography, studies in, 203-206
- Csapo, Arpad, 270, 271-272  
 studies in embryology, 19, 272, 273, 282, 283  
 publication by, 291
- Curtis, Howard J., 315



Cushner, I. M., studies in embryology, 286  
 publication by, 291, 292  
 cytology, *see* biophysics; cellular organization; ex-  
 perimental taxonomy; genetics

D

Danesino, Vittorio L., studies in embryology, 279-  
 280, 285-286  
 publications by, 291  
 Das, N. K., publication by, 332  
 Davenport, Charles B., x, 295  
 Davis, B. D., 308  
 Davis, Gordon L., ix  
 studies on age of rocks, 87-100, 161-168, 227  
 publications by, 150, 152, 228, 229  
 Day, Arthur L., ix, 190  
 De, D. N., studies on cellular organization, 315,  
 316-317  
 publication by, 332, 333  
 Dean, A. C. R., ix, 148, 152  
 De Feo, Vincent J., x, 271, 290  
 studies in embryology, 20, 286-287  
 DeHaan, Robert L., x, 271  
 Dekeyser, W., 221  
 Delano, Frederic A., vii  
 Demerec, Milislav, x, 125, 294, 296  
 report of Director of Department of Genetics, 293-  
 333  
 studies in bacterial genetics, 16, 294-295, 301-315  
 publications by, 267, 332  
 Demerec, Zlata E. (Zlata Hartman), publications by,  
 332, 333  
 derivative spectrophotometry, 256-259  
 Desert Laboratory, x  
 Des Prez, John D., 272  
 Deutsch, Armin J., ix, 60  
 stellar research, 37-38, 40, 42, 44, 55  
 publications by, 61  
 Deutsch, Dorothy S., 60  
 de Vries, Hessel, 336  
 Didusch, James F., 276  
 Djordjević, Božidar, 301-302  
 studies in bacterial genetics, 312  
 Doak, J. B., 152  
 publication by, 229  
 Dobzhansky, Th., 295  
 Dodge, Cleveland H., vii  
 Dodge, William E., vii  
 Doermann, A. H., 296  
 Donnay, Gabrielle, ix, 222  
 crystallographical studies, 203-206, 223, 226-227  
 publications by, 228  
 Donnay, J. D. H., 220  
 crystallographical studies, 223, 226-227  
 publications by, 228  
 Donoho, N. S., publication by, 291, 292  
*Drosophila Information Service*, 296  
*Drosophila* Stock Center, 296  
 Duffey, Lowell M., 274  
 publication by, 291, 292  
 Durham, Lois J., 234

E

earth's crust, studies on, 14, 71, 81-100, 154  
*See also* geophysical investigations

Ebert, James D., x, 28, 289, 290-291  
 report of Director of Department of Embryology,  
 269-292  
 studies in embryology, 18, 273-275, 278  
 publications by, 292  
 Ecklund, E. T., 152  
 studies in radio astronomy, 78-80  
 ecology, x  
*See also* experimental taxonomy; paleobotany  
 Ehrendorfer, F., publication by, 267  
 Ekelman, S. B., studies in embryology, 286  
 publication by, 292  
 electronic image converters, *see* Image Tubes for Tele-  
 scopes  
 Elliott, R. Paul, 235  
 Embryology, Department of, x, 15, 18-20, 27, 28  
 report of Director of Department of, 269-292  
 embryology, studies in, 18-20, 27, 269-292  
 England, Joseph L., ix, 222  
 geophysical investigations, 153, 154-157, 224  
 publication by, 228  
 Englesberg, E., 296  
 Enns, T., publication by, 292  
 Erickson, W. C., ix, 152  
 studies in radio astronomy, 73-78  
 upper atmosphere research, 80-81  
 Essig, S. F., 66  
 Eugenics Record Office, x  
 Eugster, Hans P., ix, 221, 222  
 studies on micas, 14-15, 153, 197-198, 218, 225  
 studies on silicate systems, 158-161, 208-210  
 studies on sulfide systems, 179-180  
 publication by, 228, 229  
 Evans, H. T., 221  
 Executive Committee, vi, XIX  
 report of the, I-XVII  
 Experimental Evolution, Department of, x  
 Station for, x  
 experimental geophysics, *see* geophysics, experimental  
 experimental taxonomy, studies in, 20, 22, 233-234,  
 236-243

F

Fairchild, William L., 234  
 Fassero, J. S., 53  
 publication by, 61  
 Faul, Henry, ix, 152  
 Feige, J., 41  
 feldspars and feldspathoids, studies on, 14, 153, 154,  
 156, 186-197  
 Fenner, Charles P., vii  
 Ferguson, Homer L., vii  
 Finance Committee, vi, I, XIX  
 Firor, John W., ix, 152  
 studies in radio astronomy, 73-78  
 upper atmosphere research, 80-81  
 publication by, 150  
 Fisher, F. J. F., 234, 235  
 studies in experimental taxonomy, 241-242  
 Flather, Edith, 45, 60  
 Fleischer, Robert, publication by, 61  
 Fleming, John Adam, ix, 23-24, 72  
 Flexner, Josefa B., 152  
 studies in biophysics, 147-148  
 Flexner, Louis B., x, 148, 152  
 studies in biophysics, 111, 147-148  
 Flexner, Simon, vii  
 Fluke, Donald, 315

- Forbes, W. Cameron, vii, I  
 Forbush, Scott E., ix, 152  
   studies in theoretical and statistical geophysics, 100-103  
   publications by, 150  
 Ford, W. Kent, Jr., 66, 67, 152  
 Forrestal, James, vii  
 Fowler, William A., 32, 42, 45  
   publication by, 61  
 Franco, R. R., 190  
 Franklin, F. A., 42  
 Franklin, K. L., ix, 152  
   studies in radio astronomy, 73-80  
 French, C. Stacy, x, 234, 235  
   report of Director of Department of Plant Biology, 231-267  
   studies on algae, 261-265  
   studies on photosynthesis, 249, 250-251, 256-259  
   publications by, 267  
 French, E. F., publication by, 150  
 Frew, William N., vii  
 Friedman, I. Irving, 217, 218, 219  
 Fry, Edward I., archaeological studies, 335  
 Fuscaldo, Kathryn E., studies on cellular organization, 315, 316, 320, 321
- G
- Gage, Lyman J., vii  
 Gardner, Ernest, studies in embryology, 275  
   publication by, 291  
 Garen, Alan, studies on bacteriophage, 300, 303  
   publication by, 333  
 Gast, Paul W., publication by, 150  
 Gates, H. S., 38  
 Gay, Helen, x, 296  
   studies on cellular organization, 17, 294, 315-316, 318-319, 320, 321  
   publications by, 333  
 Genetics, Department of, x, 15-17  
   report of Director of Department of, 293-333  
   genetics, studies in, 15-17, 293-333  
   *See also* experimental taxonomy  
   geochemistry, *see* geophysical investigations  
   Geochemistry Colloquia, 153, 221  
   geology, *see* earth's crust; geophysical investigations  
   geomagnetism, studies on, 34-35, 100-101, 103-104  
   geophysical investigations, 13-15, 153-229  
   *See also* geophysics, experimental; geophysics, theoretical and statistical  
 Geophysical Laboratory, ix, 13-15, 25, 87, 149  
   report of Director of, 153-229  
*Geophysical Research, Journal of*, 24, 72, 150  
 geophysics, experimental, studies in, 73-100  
   *See also* geophysical investigations  
 Geophysics, Theoretical, Conference on, 149, 151  
 geophysics, theoretical and statistical, studies in, 100-104  
   *See also* geophysical investigations
- Ghosh, Chitra, publications by, 332, 333  
 Giese, Arthur T., 234  
   studies on photosynthesis, 243-248  
   publication by, 267  
 Gifford, Walter S., v, vi, I, XIX  
 Gilbert, Cass, vii  
 Gilbert, Perry W., 272  
   studies in embryology, 276  
   publication by, 291
- Gillett, Frederick H., vii  
 Gilluly, James, 219  
 Gilman, Daniel Coit, vii  
 Glass, Lois, 315  
 Glickman, B. I., studies in embryology, 286  
   publication by, 292  
 Glover, Stuart W., 303  
   publication by, 333  
 Goldsmith, Julian R., 205, 219  
 Goranson, Roy W., 191  
 Gots, Joseph S., publication by, 333  
 Graham, John W., ix, 152, 220  
   studies on rock magnetism, 85-87  
   publications by, 150, 151  
 Gray, D. J., studies in embryology, 275  
   publication by, 291  
 Greenewalt, Crawford H., v, vi  
 Greenstein, Jesse L., ix, 31, 60  
   stellar and nebular research, 41-42, 44, 52  
   publications by, 61, 62  
 Greenwald, Gilbert S., x, 271  
   studies in embryology, 19, 272-273, 281-282, 287-288  
 Gregor, J. W., 238  
 Greig, Joseph W., ix, 174, 190  
 Grill, Richard D., 291  
 Grun, Paul, publications by, 267
- H
- Hale, George Ellery, ix, 22  
 Halferdahl, L. B., studies on chloritoid, 200-203  
 Hall, Harvey Monroe, 26  
 Hall, John S., 65, 66  
 Halldal, Per, x, 234  
   studies on algae, 233, 259-265  
 Hansen, John F., 234, 239  
 Hanson, A. A., 236-237  
 Hardie, R., publication by, 61, 63  
 Hardwick, E. R., 235  
 Harker, R. Ian, 218, 219, 220  
 Harper, Gordon E., 234  
   studies on photosynthesis, 256-259  
 Harrington, R. G., 33, 60  
 Hartman, Carl G., 269-270, 272  
   studies in embryology, 281  
 Hartman, Philip E., 295, 302  
   studies in bacterial genetics, 306-307, 311, 312  
   publication by, 333  
 Hartman, Zlata (Zlata E. Demerec), publications by, 332, 333  
 Hartmann, William L., studies in embryology, 286  
   publication by, 291, 292  
 Haskins, Caryl P., v, vi, xi, 337, XIX  
   Report of the President, 3-28  
   publication by, 343  
 Hass, Georg, 66  
 Hay, John, vii  
 Hayden, R. J., publications by, 151, 229  
 Heard, Osborne O., 270  
   studies in embryology, 288  
   publication by, 291  
 Helfer, H. Lawrence, ix, 152  
 Hendricks, S. B., 148  
 Henize, Karl G., 60  
   nebular research, 47  
 Henry, Barklie McKee, v, vi, I, XIX  
 Herrick, Myron T., vii

- Hershey, Alfred D., x, 148, 296  
 studies on bacteriophage, 16, 294, 297-301  
 publications by, 333
- Hertig, Arthur T., x
- Herzog, L. F., publication by, 150, 151, 229
- Hess, D., publication by, 151, 229
- Hess, Harry H., 219, 220
- Heuser, Chester H., x, 272  
 studies in embryology, 275, 288  
 publication by, 291
- Hewitt, Abram S., vii
- Heydenburg, Norman P., ix, 152  
 studies in nuclear physics, 104-110  
 publications by, 151
- Hickox, Joseph O., 60  
 solar research, 34
- Hiesey, William M., x, 234, 235  
 studies in experimental taxonomy, 26, 234, 236-239, 239-241
- Higginson, Henry L., vii
- Hiltner, W. A., 47  
 historical research, *see* archaeology
- Historical Research, Department of, x  
 Division of, x, 25, 338
- Hitchcock, Ethan A., vii
- Hitchcock, Henry, vii
- Hoffman-Berling, 290
- Holyk, W. K., publication by, 229
- Hoover, Herbert, vii
- Hopson, C. A., 220
- Howarth, Sheila, x, 301  
 studies in bacterial genetics, 312
- Howe, William Wirt, vii
- Hoyle, Fred, stellar research, 45, 49
- Hubble, Edwin P., 10, 11
- Hudson, R. P., publication by, 150, 151
- Huggett, A. St. G., 285  
 publication by, 291, 292
- Humason, Milton L., ix, 60  
 stellar and nebular research, 10, 11, 41, 48, 49  
 publications by, 61, 62
- Hume, Suzanne, 315
- Hurst, Vernon J., 220  
 crystallographical studies, 223, 226-227  
 publications by, 228
- Huster, E., 88, 91, 163
- Hutchinson, Charles L., vii
- hydrous iron silicates, *see* silicates

## I

- Image Tubes for Telescopes, report of Joint Committee on, 65-67
- Ingerson, Earl, 219
- Inghram, M. G., publications by, 151, 229
- International Geophysical Year, 81, 149
- interstellar matter and nebulae, studies on, 10-11, 11-12, 12-13, 45-53
- ionosphere, *see* upper atmosphere research
- Iriarte, B., 47
- isotope dating of ancient minerals, *see* age of rocks

## J

- Jager, C. de, publication by, 61
- James, Harold L., 220
- Jameson, J. Franklin, x

- Jeffery, P. M., 152  
 studies on age of rocks, 87-100, 161-168  
 publications by, 151, 229
- Jensen, Einar, 174
- Jessup, Walter A., vii
- Jewett, Frank B., vii
- Johnson, Ellis A., ix, 152
- Johnson, Harold L., 39, 40, 44  
 publication by, 61
- Jones, Thomas K., 56
- Joy, Alfred H., stellar research, 38  
 publication by, 62

## K

- Kacser, H., 296
- Käfer, Etta, x
- Katsh, Seymour, x, 271  
 studies in embryology, 279
- Kaufmann, Berwind P., x, 296  
 studies on cellular organization, 17, 293-294, 315-322  
 publications by, 332, 333
- Keck, David D., 26
- Keene, A. G., studies on anhydrous silicate systems, 208
- Kennedy, George C., 218, 219
- Kersh, G. R., publication by, 332, 333
- Kidder, Alfred Vincent, x, 338, 340
- King, J. C., 296
- Koski, Violet M. (Young, Violet M. K.), 249  
 publication by, 267
- Kouvo, J. A. O., 221
- Kracek, Frank C., ix, 222
- Kraft, Robert P., 55-56  
 publication by, 62
- Krauskopf, Konrad B., 218, 219
- Krauss, Robert W., publication by, 267
- Kullerud, Gunnar, ix, 221, 222  
 geophysical investigations, 15, 154, 174, 175-177, 178-183, 204-205, 224-225  
 publication by, 228
- Kupke, Donald W., x, 234  
 studies on photosynthesis, 21-22, 232, 243-248

## L

- laboratory physics, 104-148  
*See also* geophysical investigations
- Lahr, Ernest L., x  
 studies in bacterial genetics, 301, 309
- Landolt, Elias, 265  
 publication by, 267
- Langley, Samuel P., vii
- Larsen, E., Jr., publication by, 151, 229
- Larsen, Esper S., 195
- Latimer, Paul H., 234  
 studies on photosynthesis, 251-252
- Laughlin, Harry H., 295
- Lawrence, Ernest O., v, vi, XIX
- Leahy, J. J., ix, 152
- Leighton, R. B., 56
- Levinthal, C., 148
- Lewis, Harlan, x, 234, 235, 236
- Libby, Willard F., x, 153, 217, 219, 222  
 studies in radioactivity, 154, 183-186
- Lindbergh, Charles A., vii
- Lindsay, William, vii
- Lipson, H., geophysical investigations, 205
- Lodge, Henry Cabot, vii

- Loeffler, Josef E., 234  
 Lombard, R. H., 174, 180, 181  
 Loomis, Alfred L., v, vi, XIX  
 Loring, Hubert S., 235  
 Lovering, Thomas S., 219  
 Lovett, Robert A., v, vi, XIX  
 Lovtrup, Soren, publications by, 151  
 Low, Seth, vii  
 Lowen, A. Louise, 60  
 Ludovici, Bruno F., 234  
   studies on photosynthesis, 256-259  
 Luria, S. E., 295, 296  
 Lynch, Victoria H., 234  
   studies on photosynthesis, 231, 250-251, 257  
   publication by, 267
- M
- McClintock, Barbara, x  
   studies in maize genetics, 16-17, 293, 323-332  
   publication by, 333  
 McClure, F. T., ix  
 MacDonald, Gordon J. F., ix, 153, 218, 220, 221  
 McDonald, Margaret R., x  
   studies on cellular organization, 315, 320-322  
   publications by, 333  
 MacDougall, Daniel T., x  
 MacDougall, Elsie, publication by, 341  
 McDelderry, M. J., studies on cellular organization,  
   315, 320, 321  
 McGee, J. D., 66, 67  
 McHugh, Keith S., v, vi, I, XIX  
 McIntyre, Jean W., 302  
 MacKenzie, William S., ix, 221  
   geophysical investigations, 186-188, 195, 197, 205,  
   223-224, 226, 227  
   publications by, 228  
 McLaughlin, Andrew C., x  
 McNamara, D. H., 57  
 McNish, A. G., 100  
 MacRae, Donald A., publication by, 61, 62  
 MacVeagh, Wayne, vii  
 Mahler, Joy, 340  
 maize genetics, studies in, 16-17, 293, 323-332  
 Mall, Franklin P., x, 269  
 Mandel, H. George, 130, 148, 152  
 Mandell, Joseph D., x, 295  
   studies on bacteriophage, 297-301  
 Maramorosch, K., 296  
 Marcus, Silvia R., 60  
 Marton, L. L., 65  
 Mason, Brian H., 219, 220  
 Mathis, John S., 46  
 Matthews, Mildred, 41, 60  
 Matthews, Thomas A., 60  
 Maya, *see* archaeology  
 Mayall, Nicholas U., 49  
   publications by, 61, 62  
 Melechen, Norman E., x  
   studies on bacteriophage, 297-301  
 Mellon, Andrew W., vii  
 Meltzer, Allan S., 58  
 Merriam, John Campbell, vii  
 Merrill, Paul W., x  
   stellar research, 39, 42  
   publications by, 62, 343  
 Merwin, H. E., geophysical investigations, 174, 180,  
   225  
   publication by, 228, 229
- Metamorphic Facies Symposium, 153, 219-221  
 micas, studies on, 14-15, 153, 197-198, 218, 225  
 Miczaika, G. R., 42  
 Middlebrook, Mavis Joan, 272  
 Miller, Margaret Carnegie, v, vi, XIX  
 Miller, Roswell, vii  
 Miller, Stanley, 171, 172, 174  
 Miller, William C., 60  
 Mills, Darius O., vii  
 Milner, Harold W., x, 234, 235  
   studies in experimental taxonomy, 239-241  
 Minkowski, Rudolph L., ix, 59, 60  
   nebular research, 51, 55  
   publications by, 60, 62  
 Mitchell, S. Weir, vii  
 Mitchell, Walter E., Jr., solar research, 56, 57  
 Moffett, Benjamin C., Jr., 276  
   publication by, 291  
 Mohler, Orren C., 57  
 Montague, Andrew J., vii  
 Moor, J. C., 66  
 Moore, A. R., crystallographical studies, 206  
 Moore, E. C., publication by, 333  
 Morey, George W., ix, 190, 221, 222  
   geophysical investigations, 227  
   studies on vapor transport, 206-207, 218, 224, 225  
   publications by, 228  
 Morgan, Henry S., v, vi, I, XIX  
 Morgan, W. W., 44  
 Morovitz, H., 148  
 Morris, Earl Halstead, 25, 338  
 Morrow, William W., vii  
 Moser, Hermann, x, 295  
   studies in bacterial genetics, 17, 295, 301, 313-315  
   publication by, 333  
 Mottelson, B. R., 71, 104, 105, 152  
 Mount Wilson and Palomar Observatories, ix, 10-13,  
   22-23, 26, 28, 74-75, 149  
   report of Director of, 31-63  
   *See also* Image Tubes for Telescopes  
 Mudd, Seeley G., v, vi  
 Mun, A. M., 272  
   studies in embryology, 275  
 Münch, Guido, ix, 60  
   stellar, interstellar, and nebular research, 40, 41,  
   45, 47, 49, 52  
   publications by, 62  
 Münch, Luis, publication by, 62  
 Myers, William I., v, vi, XIX
- N
- National Bureau of Standards, 34, 65, 149  
 National Geographic Society-Palomar Observatory Sky  
   Survey, 13, 32-33, 46  
 Naval Observatory, U. S., 34, 65  
 nebulae and interstellar matter, studies on, 10-11,  
   11-12, 12-13, 45-53  
 Nesbitt, R. E., studies in embryology, 287  
 Nichols, Richard F. F., xi  
 Nicholson, Seth B., ix, 26, 36, 60  
   solar research, 34  
   publications by, 62  
 Nicolaysen, L. O., publications by, 150, 151, 229  
 Niu, M. C., 296  
 Nobs, Malcolm A., x, 234, 235  
   studies in experimental taxonomy, 234, 236-239



Nominating Committee, vi, I, XIX  
 Nordberg, D., 152  
 Novick, A., 296, 314  
 nuclear physics, studies in, 71-72, 104-110

## O

Olmsted, Charles E., 236  
 O'Rahilly, Ronan, studies in embryology, 275  
   publication by, 291  
 organization of cellular materials, *see* cellular organization  
 Osborn, E. F., 169, 218-219, 220  
 Osborn, William Church, vii  
 Osterbrock, Donald E., ix, 60  
   stellar and nebular research, 39, 46  
   studies on comets, 37  
   publication by, 62  
 Ozeke, H., studies in bacterial genetics, 301, 302-303, 307, 309-311  
   publication by, 333

## P

Padget, Dorcas H., studies in embryology, 275-276  
   publication by, 291  
 Padget, P., geophysical investigations, 224-225  
   publication by, 228  
 paleobiochemistry, studies in, 171-174  
 paleobotany, studies in, 265-266  
 Palomar Observatory, *see* Mount Wilson and Palomar Observatories  
 Parmelee, James, vii  
 Parsons, Wm. Barclay, vii  
 Paton, Stewart, vii  
 Patterson, C. C., 99, 100, 168  
   publications by, 150, 151, 229  
 Paul, William M., studies in embryology, 279, 286  
   publications by, 291, 292  
 Pecora, William T., 219, 220  
 Pemsler, J. P., 257  
 Pepper, George W., vii  
 Pershing, John J., vii  
 Persson, Arnulf R., 235-236  
 Pestell, Wilbur A., publication by, 267  
 petrology, statistical, studies in, 210-216  
   *See also* geophysical investigations  
 Pettijohn, Francis J., 220, 221  
 Pettit, Edison, 26  
   publications by, 62  
 Pfahler, Paul L., 234, 238  
 photometry and stellar spectroscopy, studies in, 10-12, 32-33, 37-45, 55-56, 57-59  
 photosynthesis, studies on, 20-22, 231-232, 243-256  
 phototaxis, studies on, 233, 259-261  
 physics, *see* astronomy; biophysics; Image Tubes for Telescopes; terrestrial sciences  
 physiology, *see* embryology  
 Pieper, G. F., ix  
 Pinson, W. H., Jr., publication by, 150, 151, 229  
 Pitzer, K. S., 222  
 planets and comets, studies on, 36-37  
 Plant Biology, Department of, x, 15, 20-22, 26  
   report of Director of Department of, 231-267  
 Plant Biology, Division of, x  
 plant biology, studies in, 20-22, 231-267  
   *See also* cellular organization; maize genetics  
 Plant Physiology, Laboratory for, x

Plaut, L., 37, 57  
 Pogo, Alexander, 60  
 Poindexter, E., 222  
 Poldervaart, Arie, 220  
 Pollard, E. C., 148, 152  
 Pollock, Harry E. D., x, 337  
   report of Director of Department of Archaeology, 335-342  
 Popper, Daniel M., 57  
 Posner, A. Charles, 289  
 Posnjak, Eugene, studies on anhydrous silicate systems, 209  
 Powell, Florence, 315  
   studies on cellular organization, 320, 321  
 Powers, H. A., 221  
 Pray, Thomas R., 234, 238  
 Preiss, J., 148, 152  
 Prentis, Henning W., Jr., v, vi, I, XIX  
 President, v, vi, xi, 337, I, XIX  
   Report of the, 3-28  
   publications by, 343  
 presidents, former, vii  
 pressures, extreme, studies on, 15, 153, 154-157, 218  
 primitive magmas, studies on, 168-171  
 Pritchard, R., 296  
 Pritchett, Henry S., vii  
 Proskouriakoff, Tatiana, x, 338  
   archaeological studies, 335, 336, 339-341  
   publications by, 341, 342

## R

radio astronomy, studies in, 12-13, 45, 51-53, 70-71, 73-80  
 radioactivity, studies in, 154, 183-186  
 Ramberg, Hans, x, 153, 219-220, 221  
 Ramsey, Elizabeth Mapelsden, x, 290  
   studies in embryology, 20, 284-285, 288-289  
   publications by, 292  
 range grasses, *see* experimental taxonomy  
 Rankin, G. A., 193  
 Ray, Clayton, 336, 340  
 Reber, G., 152  
 Reese, E. J., 74  
 Rehman, I. H., studies in embryology, 280  
   publication by, 291, 292  
 Reid, David G., 295  
 Rentschler, Gordon S., vii  
 research associates, x, XIX  
 Retirement Committee, vi, I, XIX  
 Reynolds, Samuel R. M., x, 270, 271, 272, 291  
   studies in embryology, 20, 279-280, 281, 285-286  
   publications by, 291, 292  
 Richardson, Robert S., ix, 60, 74  
   solar and planetary research, 34, 36  
   publications by, 62  
 Roberts, Irena Z., ix, 152  
 Roberts, Morton, publication by, 62  
 Roberts, Richard B., ix, 82, 152, 305  
   studies in biophysics, 110-148  
   publications by, 150, 151  
 rock magnetism, studies on, 71, 85-87  
 Rockefeller, David, vii, XIX  
 Roedder, Edwin W., 220, 221  
 Rogers, Oliver V., Jr., 295  
 Rogerson, John B., Jr., 60  
 Roman, H., 296  
 Root, Elihu, vii

- Root, Elihu, Jr., v, vi, XIX  
 Root, William C., 336, 340  
 Rosenwald, Julius, vii  
 Rowland, W. R., 222  
 Roy, Rustum, 219  
 Roys, Ralph L., 341  
 Rubey, William W., 171, 172, 218, 219, 220  
 Rule, Bruce, 53, 60  
   publication by, 61, 62  
 Ruppert, Karl, x  
   archaeological studies, 336  
   publications by, 341, 342  
 Ruth, Royal F., x, 271  
   studies in embryology, 275  
   publication by, 292  
 Ryerson, Martin A., vii

## S

- Sahade, Jorge, 57-58  
   publication by, 62  
 Sandage, Allan R., ix, 60  
   stellar and nebular research, 38, 39, 40, 48, 49, 58  
   publications by, 61, 62, 63  
 Sanders, William T., archaeological studies, 337  
   publication by, 342  
 Sanford, Roscoe F., stellar and interstellar research,  
   39, 42, 47  
   publication by, 63  
 Sang, J. H., 296  
 Sarton, George Alfred Leon, 24-25, 338  
 Schairer, J. Frank, ix, 221, 222-223, 227  
   geophysical investigations, 190, 195-197, 202, 205,  
   207-210, 211, 223, 224, 225-226  
   publications by, 228  
 Scherer, Paul A., xi  
 Schmidt, M., 152  
 Schmidt, Maarten, 60  
 Schofield, Brenda M., studies in embryology, 282-  
   283  
   publication by, 292  
 Schrift, Alex, 148, 152  
 Schwarzschild, M., publication by, 61, 63  
 Scott, Roger B., 289  
 Scott, W. E., publications by, 151  
 Seaton, M. J., 46  
 Seegar Jones, Georgiana E., publication by, 292  
 seismic studies, 14, 71, 81-85, 153, 154  
 Sensenig, E. Carl, studies in embryology, 276  
   publication by, 291  
 Shain, C. A., 37, 75  
 Shepard, Anna O., x  
   studies in ceramic technology, 337  
   publication by, 341, 342, 343  
 Shepley, Henry R., v, vi, XIX  
 Shibata, Kazuo, 234  
   studies on photosynthesis, 21, 231-232, 248-250,  
   252-256  
 Shook, Edwin M., x, 337, 338  
   archaeological studies, 336-337  
   publications by, 341, 342  
 Shryock, Richard H., 269-270  
 silicates, studies on, 154, 158-161, 207-210  
 Simpson, John, 149  
 Singleton, F., 257  
 Skaar, P. D., 296  
 Smith, A. Ledyard, x  
   archaeological studies, 336  
   publications by, 341, 342, 343  
 Smith, Derek A., x, 295, 302  
   studies in bacterial genetics, 303-304  
 Smith, Edwin Ide, studies in embryology, 276-277  
   publication by, 291  
 Smith, Guinevere C., 296  
 Smith, J. R., 221, 223  
   geophysical investigations, 186, 188-194, 195-197  
 Smith, J. V., geophysical investigations, 195, 203,  
   223-224, 226, 227  
   publications by, 228, 229  
 Smith, James H. C., x, 234, 235, 236  
   studies on photosynthesis, 21-22, 232, 243-248,  
   249, 251  
   publications by, 267  
 Smith, Robert E., x, 337  
   archaeological studies, 335, 336, 338-339  
   publications by, 341, 342  
 Smith, Sydney, 315  
 Smith, Theobald, vii  
 Snyder, Emmy M., 302  
 solar research, 33, 34-36, 56-57  
   *See also* radio astronomy  
 solubility of solids in steam, *see* vapor transport  
 spectroscopy, *see* nebulae and interstellar matter;  
   stellar spectroscopy and photometry  
 Spiegelman, S., 148  
 Spitzer, Lyman, 59  
 Spoehr, Herman Augustus, x  
   publication by, 267  
 Spooner, John C., vii  
 Stebbins, Joel, 49, 55  
 Steggerda, Morris, 295  
 Steinberg, Malcolm S., x, 271  
 stellar evolution, studies on, 11-12, 31-32, 39, 40-  
   41, 42, 43, 44-45  
 stellar spectroscopy and photometry, studies in, 10-12,  
   32-33, 37-45, 55-56, 57-59  
 Sternglass, E. I., 66  
 Stewart, David B., ix, 220, 222  
   geophysical investigations, 190-195  
 Storey, William Benson, vii  
 Stoudenheimer, R. G., 66  
 Streeter, George L., x, 269, 272, 275, 277  
 Strehler, Bernard L., x, 234  
   studies on algae, 232-233  
   studies on photosynthesis, 248, 251, 257  
   publication by, 267  
 Streisinger, George, x, 295  
 Stroebel, C. F., 148, 152  
 Strömsvik, Gustav, x, 337  
   archaeological studies, 336, 338  
 Strong, Richard P., vii  
 Struve, Otto, 56, 57-58  
   publication by, 62, 63  
 Stryker, Lucile B., xi  
 Sugiura, M., 152  
 sulfide systems, studies on, 15, 174-183  
 Sullivan, James F., xi  
 Suttle, A. D., Jr., studies in radioactivity, 183  
 Swanson, Oscar, 54, 60  
 Swift, Dorothy R., xi  
 Swope, Henrietta H., 60  
   nebular research, 47-48  
 Szilard, Leo, 314

## T

- Taft, Charles P., v, vi, XIX  
 Taft, William H., vii  
 Tait, D. B., 169  
 Tatel, Howard E., ix, 152  
   seismic studies, 14, 81-85  
   studies in radio astronomy, 78-80  
   publications by, 151  
 taxonomy, *see* experimental taxonomy; paleobotany  
 Taylor, R. J., 58  
 TeLinde, Richard W., 289  
 Temmer, Georges M., ix, 152  
   studies in nuclear physics, 104-110  
   publications by, 150, 151  
 Temple, Charles R., publication by, 341, 342  
 Terrestrial Magnetism, Department of, ix, 13-14, 15,  
   23-24, 36-37, 65, 153, 161, 305  
   report of Director of Department of, 69-152  
 terrestrial magnetism, studies on, *see* geomagnetism  
*Terrestrial Magnetism and Atmospheric Electricity,*  
*Journal of*, 24, 72  
 terrestrial sciences, ix  
   Committee on, vi  
   studies in, 13-15, 69-229  
 Terry, Sarah, 315  
 Thayer, William S., vii  
 theoretical geophysics, *see* geophysics, theoretical and  
   statistical  
 Thomas, William H., publication by, 267  
 Thompson, Donald E., publications by, 341, 342  
 Thompson, J. Eric S., x, 28, 338  
   archaeological studies, 336  
   publications by, 341, 342  
 Thompson, James B., Jr., 218, 219, 220, 221  
 Thurston, R. K., 295  
 Tift, William G., 38  
 Tilley, C. E., x, 153, 197, 219, 220, 221, 222  
   geophysical investigations, 169-171, 182  
 Tilton, George R., ix, 152, 222  
   studies on age of rocks, 87-100, 161-168, 227  
   publications by, 150, 151, 152, 228, 229  
 Trippe, Juan T., v, vi, XIX  
 Trustees, Board of, v, 70, I  
   Abstract of Minutes of, XIX  
   committees of, vi  
   former, vii  
 Tunell, George, 219  
 Turner, Francis J., 219, 221  
 Tuttle, O. Frank, 219, 221, 222  
   geophysical investigations, 182, 186-187, 191, 211,  
   212, 214, 218  
 Tuve, Merle A., ix, 72, 152  
   report of Director of Department of Terrestrial  
   Magnetism, 69-152  
   report of Joint Committee on Image Tubes for  
   Telescopes, 65-67  
   seismic studies, 81-85  
   studies in radio astronomy, 78-80  
   publications by, 151  
 Tuve, T. W., 82, 148

## U

- United States history, section of, x  
 upper atmosphere research, 80-81

## V

- Vannevar Bush Symposium on the Role of Volatiles  
   in Geological Processes, 153, 217-219  
 van Wijk, Uco, 58  
 vapor transport, studies on, 206-207, 218, 224, 225  
 Venkatesh, Chakraborti S., x, 234  
   studies in experimental taxonomy, 236, 239  
 Verhoogen, John, 218, 219  
 Vestine, Ernest H., ix, 152  
   studies on geomagnetism, 103-104  
   publications by, 150, 151  
 Vielmetter, Walter, studies in bacterial genetics, 301,  
   307-308  
 Virgin, Hemming I., 262  
   publication by, 267  
 Vogel, N. W., 272  
 Vokes, F. M., geophysical investigations, 224-225  
   publication by, 228-229  
 von Borstel, R. C., publications by, 333  
 von Hoerner, Sebastian, 58  
 von Neumann, John, x

## W

- Wachtel, M. M., 66  
 Wadsworth, James W., vii  
 Wagenaar, Sibergina, 295, 315  
   studies on cellular organization, 322  
 Wahlquist, Hugo D., 40  
   publication by, 61, 63  
 Walcott, Charles D., vii  
 Walcott, Frederic C., vii  
 Walcott, Henry P., vii  
 Walker, Merle F., stellar research, 37, 38, 40, 59  
   publications by, 63  
 Wallace, B., 296  
 Wallerstein, G., 40  
 Ward, George H., x, 234, 235  
   studies in experimental taxonomy, 242-243  
 Wasserburg, G. J., publication by, 229  
 Waters, Aaron C., 219, 220, 221  
 Watson, Ernest C., 31  
 Watson, Patricia, 238  
 Weber, H. H., 290  
 Weed, Lewis H., vii  
 Welch, William H., vii  
 Wells, Harry W., ix, 152  
   studies in radio astronomy, 73-78  
   upper atmosphere research, 80-81  
   publication by, 151  
 Went, Frits W., 236  
 Westergaard, Mogens, 236  
 Wetherill, George W., ix, 152, 221, 222  
   studies on age of rocks, 87-100, 161-168, 227  
   publications by, 150, 151, 152, 228, 229  
 Wharton, Lawrence R., Jr., 272, 289  
 Wheeler, Hilda H., 295  
 White, Andrew D., vii  
 White, Donald E., 217, 219  
 White, Edward D., vii  
 White, Henry, vii  
 White, James Norman, v, vi, XIX  
 White, Walter S., 220, 221  
 Whitford, A. E., 49, 55  
 Whiting, F. B., publication by, 229  
 Wickersham, George W., vii  
 Wiggins, Ira L., 236

Wild, Paul E., 60  
    publication by, 63  
Wilkie, Douglas, 272  
    studies in embryology, 283  
Wilkinson, D. H., 152  
Willier, B. H., 274  
Wilson, Albert G., 32-33  
Wilson, Olin C., ix, 60  
    stellar and nebular research, 38, 39, 44, 45, 55, 58  
    publications by, 62, 63  
Wilson, Ralph E., 79  
Wilson, Robert E., v, vi  
Wilt, Fred, 271  
Winters, Howard D., publications by, 341  
Witkin, Evelyn M., 296  
Woese, C., 148, 152  
Wolff, John B., 235-236  
Wood, J. L., publication by, 292  
Woodbury, Richard B., 340  
Woodward, Robert Simpson, vii  
Wright, Carroll D., vii  
Wright, Fred. Eugene, 193  
Wulf, Oliver R., 34  
    publication by, 62, 63  
Wyttanbach, Charles, 271

## Y

Yagi, K., geophysical investigations, 202  
Yanofsky, Charles, 296, 302  
    studies in bacterial genetics, 308, 309  
Yoder, Hatten S., Jr., ix, 222, 223  
    geophysical investigations, 15, 154, 169-171, 181-183, 190-194, 195, 198, 201, 203, 208, 218, 223, 225  
    publications by, 229  
Young, Violet M. K. (Koski, Violet M.), 249  
    publication by, 267  
Yura, Takashi, 302  
    studies in bacterial genetics, 308-309, 311  
    publications by, 333

## Z

Zebergs, V., publication by, 62, 63  
Zies, E. G., geophysical investigations, 225  
    publication by, 229  
Zinder, Norton, 302, 303  
    publication by, 333  
zoology, *see* embryology; genetics  
Zwicky, Fritz, ix, 60  
    stellar and nebular research, 13, 38, 41, 50, 51  
    publications by, 63

















